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PHYTOPATHOLOGY

VOLUME 19 NUMBER 8

AUGUST, 1929

ETIOLOGY OF THE PINK-ROOT DISEASE OF ONIONS

H. N. HANSEN

INTRODUCTION

The name "pink root" appeared in the literature for the first time in 1917 (7), when Taubenhaus published a preliminary report followed by partial descriptions of the disease in 1918 and 1919 (8, 9) and a full account of extensive experiments on etiology and control in 1921 (10). He considered the causal agent to be a species of Fusarium and named it *F. malli* Taub. Sideris (5, 6) concluded that the disease was caused by several species of Fusarium and not by *F. malli* alone.

ECONOMIC IMPORTANCE

Definite data on economic losses from the pink-root disease are not available but it is a common observation in California that where onions are grown in the same field for two or three seasons the crop frequently is so diminished that no attempt is made to harvest it. Such conditions are commonly observed in the principal onion-growing sections of the State, in the vicinity of Stockton.

SYMPTOMS

Macroscopic Symptoms. The affected roots are characteristically pink, the shade varying with the severity and age of infection. Thus, shortly after infection has taken place, the color of the roots is a rhodonite pink (3) which, as the disease progresses, deepens through the various shades of pink to spinel red and rarely to Tyrian purple. In early stages the pink color alone is the criterion of infection but, as the disease progresses, the roots lose their turgidity and assume a semi-transparent, water-soaked appearance. At this stage, the epidermal layer and the vascular elements may be easily separated as all the intervening tissues have been disintegrated. When roots are in this condition they are easily invaded by other members of the soil flora but especially by bacteria and many species of Fusarium, as shown by the writer's[1] early isolations and by those of Sideris (6).

[1] Since the above article was accepted for publication Sideris' thesis (6) has appeared in this journal, 19: 233–268. The second paragraph on page 233 does not mention the writer's preliminary publication (2) on the subject. The fact that Sideris'

Where most of the roots of a plant are infected and have reached the stage just described, the above-ground symptoms are distinctly those of starvation or drought. These symptoms, etiolation, flaccidness, wilting and dying back from the tip, are not specific for the pink-root disease, as they may occur in other diseases or under environmental conditions unfavorable to the host. Likewise the nipple-shape basal plate, mentioned as a symptom by Taubenhaus (10), is not specific for pink root, as it is not always found on infected onions and frequently is found on noninfected ones. In cases of severe infection, the general effect on the host is that of stunting the bulbs which are below a marketable size.

Microscopic Symptoms. On microscopic examination, the root tissues are found to be invaded by a fungus mycelium which often goes up into the root bases imbedded in the bulb plate, but it has not been found in the basal plate proper. The pigment which gives to the infected roots their characteristic pink color diffuses out, or is produced, ahead of the fungus filaments, sometimes to a distance of ten to twelve cells. In certain cells, sometimes close together, sometimes far apart, the mycelium accumulates, swells into various shapes, and turns dark in color. This phenomenon is the beginning of pycnidium formation and, though it may be observed in all parts of the root, it is seen more frequently in the epidermal cells.

The writer engaged in the pink-root problem with the object of determining how many and what species, varieties, or strains of Fusarium are able to produce the disease and which of these, singly or in combination, are most virulent. At the beginning of this work (Dec., 1925) no fresh material was available. Consequently, the preliminary isolations were made from the root remains and bulb plates of dry onions which had been grown in a pink-root infected field at University Farm, Davis, California. These onions still showed unmistakable evidence of having been affected with pink root. For isolation purposes material of this kind is quite undesirable because it absorbs any liquid disinfectant used and surface sterilization of the tissues can therefore not be practiced. Hence many organisms other than the pathogens are invariably obtained. The following method of isolation was used: Small pieces of root and bulb plates were washed with sterile water and placed on nutrient agar in Petri dishes, four pieces in each of twenty plates, giving eighty plantings. From the edges of the resultant colonies more than three hundred transfers were made, yielding many species of Fusarium and other members of the soil flora. From the

work was done prior to 1924 and not prepared for publication until several years later probably explains this omission. At the top of page 235 is listed a number of organisms which were isolated, among these a Phoma. The writer was unable to give credit in his article for this find, since Phoma is not mentioned in the thesis deposited in the University of California library.

Fusarium cultures more than two hundred single-spore isolations were made by the capillary-tube method (1). From these isolations there were obtained the following species of Fusarium which were identified by the usual method of parallel cultures on various media: *F. malli, F. cromyopthoron* Sid., *F. rhizochromatistes* Sid., *F. moniliforme* Sheld., and ten additional species, varieties, and strains of the same genus. The latter were not specifically identified but for inoculation purposes labelled F. 1 to F. 10. The isolated fungi were used in the following experiment:

Two hundred and thirty two-inch pots were filled with rich greenhouse soil and steamed for four hours at twenty pounds pressure. Three days after steaming, the soil from five of the pots was cultured on nutrient agar and found sterile; the remaining 225 pots were then inoculated with the isolated fungi as shown in table 1. The inoculum was taken from twenty-day-old cultures on potato slants and worked well into the soil with sterile spatulas. In order to give the fungi a good start the pots were left five days longer before each was planted with three onion seeds of the Yellow Globe Danvers variety. The seeds were treated for 10 minutes with mercuric chloride 1:500 and rinsed with sterile water. The experiment was carried on in the greenhouse at temperatures varying between 25 and 28° C. From time to time after germination plants were removed from the pots and examined for symptoms of pink root. The experiment was terminated forty days after germination when all the seedlings were removed and examined. At that time all the remaining seedlings had well-developed

TABLE 1.—*Results of growing onion seedlings in Fusarium-inoculated soil*

No.	No. pots	No. seedlings	Inoculum	Pink root
1	15	38	*F. malli*	none
2	15	42	*F. cromyopthoron*	"
3	15	42	*F. rhizochromatistes*	"
4	15	40	*F. moniliforme*	"
5	15	37	F. 1	"
6	15	36	F. 2	"
7	15	39	F. 3	"
8	15	33	F. 4	"
9	15	42	F. 5	"
10	15	42	F. 6	"
11	15	45	F. 7	"
12	15	36	F. 8	"
13	15	39	F. 9	"
14	15	39	F. 10	"
15	15	38	none (controls)	
Totals	225	588		None

root systems but were somewhat crowded in the small pots. The results of the experiment are given in table 1. Seeds planted December 19, 1925. Experiment terminated February 10, 1926.

As shown in table 1, the results were entirely negative; no pink root or other abnormalities of the roots were observed. In view of the positive results obtained by Taubenhaus (10) and by Sideris (6) it seems probable that in my work the organisms to which the disease was attributed had not been used. Hence supposedly authentic cultures of *F. malli* and *F. cromyopthoron* were obtained (the culture of *F. malli* was one Sideris had been working with at Berkeley and the culture of *F. cromyopthoron* was sent sent to me by him from Hawaii), and the inoculation experiment repeated on 24 plants; 6 inoculated with *F. malli*, 6 with *F. cromyopthoron*, 6 with both, and 6 left uninoculated as controls. Both Sideris and Taubenhaus agree that high temperatures and other conditions unfavorable to the host favor the development of pink root. During the present experiment temperatures were kept between 28° and 32° C., water was applied in varying amounts at irregular intervals, and the root systems of the inoculated plants were mutilated by cutting down through the soil with a knife. Two months later there was no evidence of pink root or other disease symptoms, indicating that either some important factor had been overlooked or that the supposedly authentic cultures of Fusarium were not pathogenic to onions. At this time (May, 1926), work with the fungi isolated from dry onions was discontinued, new isolations were made from infected roots of young, growing onions obtained from the following places: Stockton, Sacramento, the Imperial Valley, and University Farm, Davis. Culturing the fresh material gave results somewhat at variance with those previously obtained from dry onions, a result no doubt due to the fact that surface sterilization could be practiced on this material. The new isolations were made by immersing pieces of infected roots in mercuric chloride 1:500 for three minutes, rinsing in sterile water to remove excess fungicide, and culturing on Czapek's nutrient agar. Roots in advanced stages of the disease yielded a few species of Fusarium and a species of Phoma. Roots in the very early stages, when still turgid and firm, though distinctly pink, yielded no Fusaria but only a species of Phoma. The fact that the latter organism occurred on diseased material from all of the above-named places indicated that it might be an important factor in the pink-root disease.

In view of the results obtained by myself and previous investigators special precautions were taken to have the pathogenicity test of this new fungus conducted under as nearly aseptic conditions as possible. The bottom of each of ten 500 c.c. Erlenmeyer flasks was covered with about four centimeters of clean sand wetted with nutrient solution. The flasks

were stoppered with cotton plugs and autoclaved for three hours at twenty pounds pressure. After they had cooled, eight of the flasks were inoculated with *Phoma* sp. and two left uninoculated as controls. The inoculum was taken from twenty-day-old cultures on Czapek's agar and consisted of about ten grams of mycelium and medium. Each of the ten flasks was then planted with four onion seeds (Yellow Globe Danvers) which had been immersed in mercuric chloride 1:500 for fifteen minutes. Ten days after germination, many of the seedlings in the inoculated flasks showed typical symptoms of pink root and in four weeks all were dead. The controls remained healthy. Upon culturing the onion remains and sand from the inoculated flasks the fungus was obtained in pure culture. Culturing the seedlings and sand from the controls gave no cryptogamic flora whatever. The above experiment was repeated with rooted onion bulbs as follows: 24 onion bulbs (Ebenezer variety) were immersed in a 0.5 per cent solution of Semesan for 30 minutes, and placed on bottles containing a nutrient solution. When the roots were three inches or more in length the cultures were inoculated as follows: 6 with *Phoma* sp., 6 with *Fusarium malli,* 6 with *Fusarium cromyopthoron,* and 6 left uninoculated as controls. The Fusarium inocula were from three different sources, viz: 1, isolated from root remains of dry onions; 2, isolated from fresh onion roots, and 3, ob-

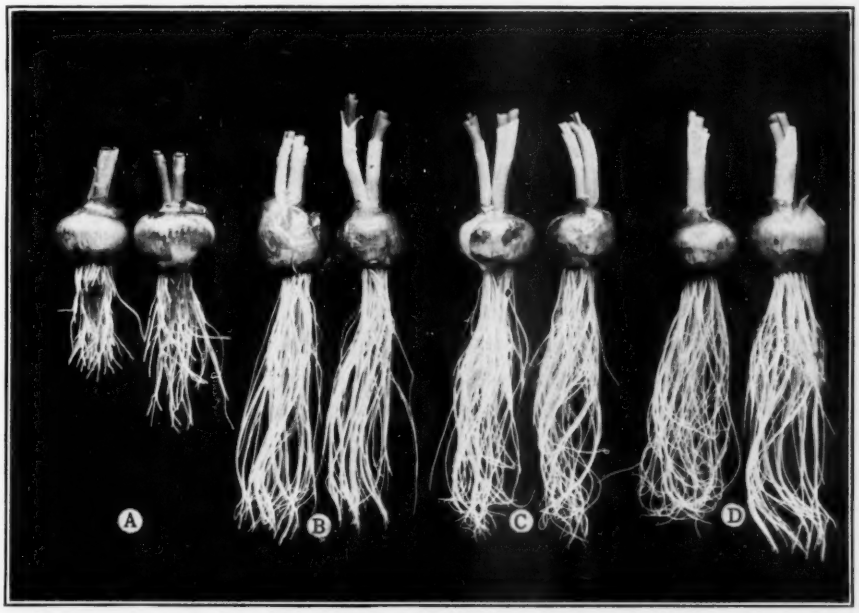

FIG. 1. A. Inoculated with *Phoma* sp. B. Inoculated with *Fusarium cromyopthoron.* C. Inoculated with *Fusarium malli.* D. Controls.

tained from Sideris. Each inoculum, consisting of about five grams of fungus and medium, was floated on the surface of the nutrient solution in contact with the roots. The experiment was initiated May 28, 1926, and terminated July 18, 1926. The results are given in table 2 and illustrated in figure 1.

TABLE 2.—*Comparative parasitism on onions of Phoma sp. and Fusarium spp.*

Culture No.	Inoculum	Pink Root
1– 6	*Phoma* sp.	all infected
7– 8	*Fusarium malli* No. 1	none "
9–10	" " " 2	" "
11–12	" " " 3	" "
13–14	*F. cromyopthoron* No. 1	" "
15–16	" " " 2	" "
17–18	" " " 3	" "
19–24	Control	" "

The results of the previous experiments indicate that the cause of pink root in California is *Phoma* sp. rather than *Fusarium malli*, *Fusarium cromyopthoron*, or any other species of that genus. It would seem very likely, however, that species of Fusarium might act as secondary parasites and thus contribute to the virulency of the disease. The following experiment was devised to test this possibility: 240 half-pint milk bottles were filled with washed sand, wetted with a nutrient solution, and autoclaved for four hours at twenty pounds pressure. The bottles were then inoculated as follows: 100 inoculated with *Phoma* sp. plus *Fusarium* spp. (consisting of a mixture of spores from more than fifty cultures of *Fusarium* comprising the isolations made earlier from both dry and fresh material and from identified cultures of *F. malli*, *F. cromyopthoron*, and *F. rhizochromatistes*). The inocula were applied as spore suspensions in sterile tap-water. Forty bottles were left uninoculated as controls. After inoculation an onion bulb (Ebenezer variety), treated for 30 minutes with 0.5 per cent solution of Semesan, was placed on each bottle. For the first six weeks of the experiment no difference could be detected between the onions inoculated with *Phoma* sp. alone and those inoculated with both *Phoma* sp. and *Fusarium* spp. After that time, however, the latter proceeded to go down more rapidly and at the time the experiment was concluded (90 days after inoculation) nearly all were dead, while those inoculated with *Phoma* sp. only were still alive (Fig. 2). Upon microscopic examination it was found that in the onions inoculated with both *Phoma* sp. and *Fusarium* spp. the bulb plates and, in most cases, the bulbs themselves were heavily infected with mycelium of Fusarium. Where *Phoma* sp. alone had been used as

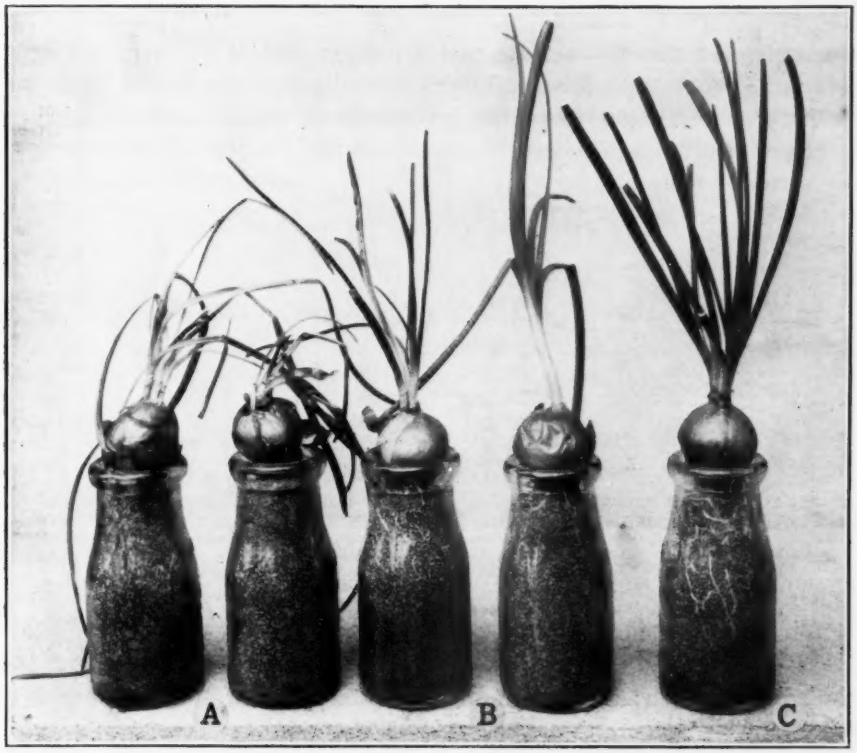

FIG. 2. A. Inoculated with *Phoma* sp. and *Fusarium* spp. B. Inoculated with *Phoma* sp. C. Control.

inoculum the bulbs and bulb plates were not invaded. Results of the above experiment indicate that where the host has already been invaded by a parasite, species of Fusarium are able to enter and materially aggravate the diseased conditions.

TEMPERATURE RELATIONS

The following experiment was designed to determine the optimum temperature for infection of onion roots by *Phoma* sp. Twenty-four shallow flower pots, 6 cm. deep by 30 cm. in diameter, were filled with loam soil and autoclaved at 20 pounds pressure for four hours on three successive days. Each of these pots was heavily inoculated with cultures of *Phoma* sp. and planted with five Ebenezer onion bulbs which had been immersed in a 0.5 per cent solution of Semesan for 30 minutes. Four pots were placed in each of six constant temperature chambers. As none of these chambers were equipped for illumination it was necessary to use onion bulbs rather than seedlings. Experimenting with plants grown from bulbs has the additional advantage that, in them, roots and tops can develop for a con-

siderable length of time independent of outside sources of energy or food materials, and also to a certain extent independent of one another; hence, infected roots would not be expected materially to affect growth of the tops and, therefore, temperature effect on growth of the host and on degree of infection could be studied in the same plants. Results of the experiment are given in table 3.

TABLE 3.—*Effect of temperature on pink-root development*

No.	Temperature	No. of bulbs	Plant development[1]	No. of plants infected	Per cent infected
1	4 C.	20	10	0	0.0
2	10 C.	20	30	0	0.0
3	13 C.	20	40	1	5.0
4	20 C.	20	80	15	75.0
5	25 C.	20	100	20	100.0
6	30 C.	20	70	4	25.0

[1] Rather than classify plant development as very poor, poor, fair, etc., a numerical classification was adopted by arbitrarily giving the lot of plants with greatest growth the rating of 100 and measuring the others by this standard.

It will be seen from the foregoing results that apparently no infection takes place at 4° and 10° C. and but little at 13° C. These temperatures, however, are far too low for normal development of the host. As indicated in table 3, the highest percentage of infection coincides with the greatest growth of the plants.

TABLE 4.—*Growth of Phoma sp. at various temperatures*

Temperature in degrees C.	4 days	8 days	12 days	16 days	20 days
	Diameter of colonies in mm. at indicated intervals				
4	0	0	0	0	0
10	0	0	0	0	0
13	0	3	5	8	12
20	10	26	42	59	76
24	20	38	56	76	90[1]
25	21	40	59	78	90
26	22	42	63	84	90
28	20	37	56	75	90
30	17	34	51	70	85
32	9	20	32	44	56

[1] Plate overgrown.

In order to correlate the effect of temperature on infection and on maximum growth of the fungus, fifty plates, each containing 10 c.c. of corn-meal agar, were inoculated with spores of *Phoma* sp. and incubated at constant temperatures. Five plates were placed at each temperature and the colonies measured at four-day intervals. The measurements given in table 4 are averages of five colonies.

It will be observed from the above results that *Phoma* sp. grows very slowly at the lower temperatures and that the optimum is not far from 26° C. Comparing the results in tables 3 and 4, we find that the optimum temperature for infection of onion roots is also optimum for best growth of *Phoma* sp. in culture and perhaps also optimum for host development.

THE PATHOGEN

In reading descriptions of the thousands of species of Phoma in Saccardo's Sylloge Fungorum (4) and in other mycological works, one can not fail to notice how closely many of the species resemble one another morphologically. Seemingly habitat or host has in many instances served as the only basis of separation. It might, therefore, seem unwise to encumber the literature further by adding a new species to a genus already so numerously represented. However, in view of the fact that the newly isolated fungus differs considerably morphologically and culturally from the comparatively few other species of that genus described as root parasites, I think that its recognition is justifiable and suggest for it the name **Phoma terrestris,** n. sp.

Type Morphology. Pycnidia subglobose, ostiolate, papillate, dark brown to black, carbonaceous, 170 to 350 μ, may occur singly, frequently gregarious (Fig. 4). Conidia continuous, hyaline, oblong-ovoid, 4.5–5.5 × 1.8–2.3 μ biguttulate, sessile in pycnidium, escaping as a gelatinous mass through ruptures rarely as a gelatinous cirrus through the ostiole. Mycelium septate, hyaline, guttulate 1.0–4.5, frequently anastomosing.

On Czapek's, Conn's, Board of Health, Pfeffer's, and other synthetic media of the same type, there is very little pycnidium formation and, as a study of the physiology of the fungus was not intended, they were little used. The colors produced in these media are essentially the same as those occurring in invaded onion roots with the exception that in very old cultures, 3 months or more, the color often becomes nearly black, due to darkening of the mycelium. Corn-meal agar was found to be well suited to the formation of both pycnidium and pigment. On this medium the color varies between deep rose pink to rose lane purple in old cultures. If spore transfers are made to this medium, pycnidia usually are formed in from 3 to 5 days; if transfers are made from mycelium, pycnidia usually do not begin to form for from 10 days to 3 weeks. The capacity of *Phoma terrestris* to form pycnidia becomes markedly greater by making transfers from spores only and also

markedly less if mycelial transfers are repeatedly made (Fig. 4). One of my cultures (Fig. 4, A), derived from repeated mycelial transfers, has been grown on corn-meal agar for 60 days without producing pycnidia. This is not a phenomenon peculiar to Phoma, as Wineland (12) and several other investigators have observed similar behavior in species of Fusarium. Pycnidium formation apparently begins by swelling and lateral division of hyphal cells, giving rise to conglomerate masses of dark thick-wall bodies somewhat resembling chlamydospores (Fig. 3). These bodies are always

FIG. 3. *Phoma terrestris*, n. sp. Beginning of pycnidium formation in corn-meal agar. Similar bodies are formed within the host cells.

found in the cells of infected onion roots, where mature pycnidia apparently never occur. Here the bodies reach about the same stage of development obtained in media containing comparatively large amounts of free sugars, which seem to be a deterrent to the formation of mature pycnidia. It is likely that they serve as a carry-over or resting stage of the fungus. The mature pycnidia vary considerably not only in size, as indicated in the technical description, but also in shape, papillation, and in length and number

of the ostioles. The cultural differences of the various isolations shown in figures 4 and 5 might be deemed of varietal magnitude. Considering, however, the derivation of A and B from C of figure 4, and, further, considering the fact that all the variants are able individually and collectively to cause the pink-root disease of onions, it is the writer's opinion that all the variants should be classified as strains of *Phoma terrestris.*

Distribution. As previously stated, *Phoma terrestris* has been found on onion roots from various parts of the State and the writer maintains that it, or strains of it, constitute the cause of pink root of onions everywhere. Shortly after my first publication in 1926 (2), E. L. Felix, of the Plant Pathology Division at Cornell University, sent me a culture of a fungus

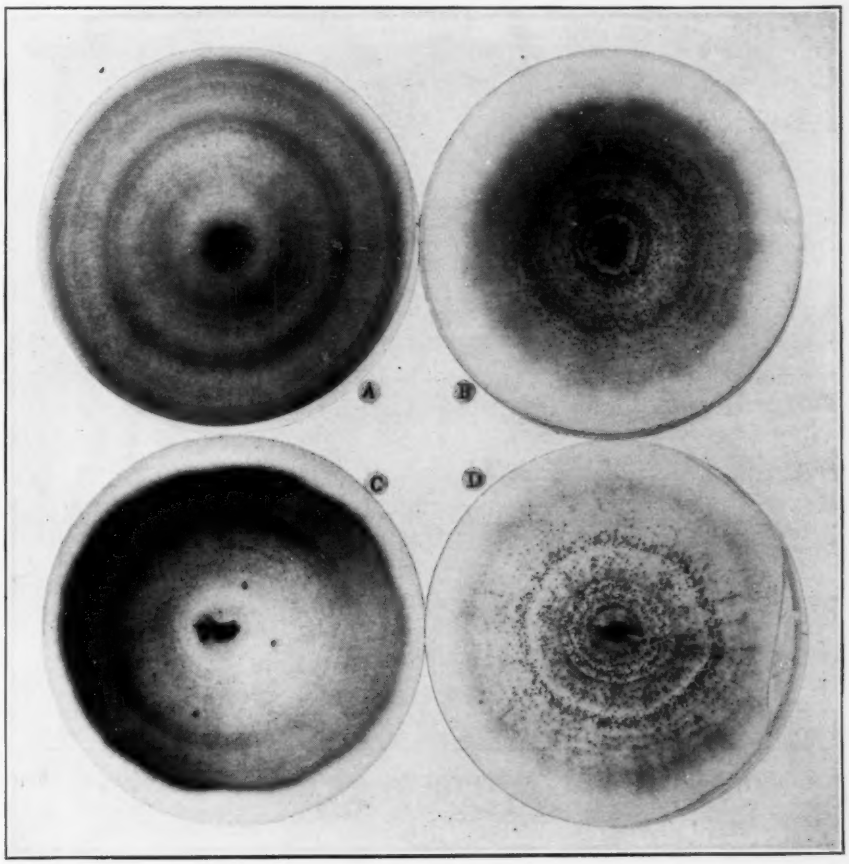

FIG. 4. *Phoma terrestris*, n. sp. A. Derived from C by constant mycelial transfers. B. Derived from C by constant spore transfers. C. Original isolation from diseased onion roots (California). D. Isolated from diseased onion roots (Wisconsin).

with which he had been able to produce pink root of onions. This organism appeared identical with mine except that it did not produce mature pycnidia; later, when Mr. Felix was in Bermuda, he graciously sent me some preserved material from there and, though this material could not be cultured, microscopic examination revealed the presence of mycelium similar to that of *Phoma terrestris* or a closely related species. In 1927, I received several cultures of Phoma from L. F. Alexander, stationed at Madison, Wisconsin. In a letter Mr. Alexander states that he invariably failed to pro-

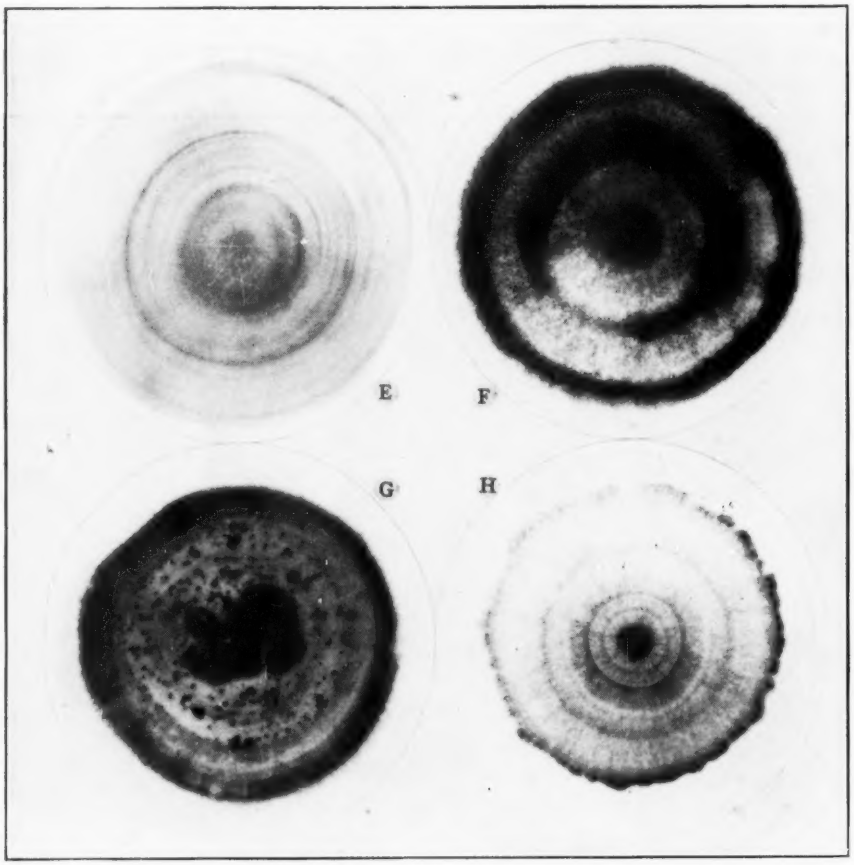

FIG. 5. *Phoma terrestris*, n. sp. E. Isolated from potato seed piece (California). F and G. Isolated from diseased onion roots (Texas). H. Isolated from diseased onion roots (New York).

duce pink root of onions with species of Fusarium but that he always obtained positive results when inoculating with the Phoma-like fungi. Of the cultures sent by Mr. Alexander, one was identical with that obtained from

Felix, one apparently identical with mine, and one differing slightly in abundance of pycnidium formation.

In 1928, I isolated two strains of *Phoma terrestris* from pink-root-infected onions grown in Bexar County, Texas (Fig. 5, F and G). I have also isolated the fungus from lesions on the roots of cowpea, *Vigna sinensis* Endl., lima bean, *Phaseolus limensis* Macf., and from potato seed pieces. The importance of the fungus as a pathogen on these and other hosts is being further studied. The above indicates that *Phoma terrestris* is not confined to members of the genus Allium but that it is a common soil habitant of wide distribution, capable of attacking the roots of quite divergent genera of plants.

SUMMARY

1. The disease known as pink root of onions has been further studied and described. The work of previous investigators is briefly discussed. They conclude that the disease is caused by species of Fusarium.

2. Inoculation experiments in sand, soil, and water cultures under various conditions with *Fusarium malli, F. cromyopthoron, F. rhizochromatistes,* and ten other species, strains, and varieties of Fusarium gave negative results, indicating that, as far as California is concerned, members of the genus Fusarium are not responsible for the pink-root disease.

3. A fungus belonging in the genus Phoma was isolated from living onion roots affected with the pink-root disease. Inoculation experiments with this fungus under controlled conditions invariably gave positive results, indicating that it is the true causal agent. The new fungus is described and the name **Phoma terrestris,** n. sp., suggested.

4. Inoculation experiments with both *Phoma terrestris* and *Fusarium* spp., seem to show that species of Fusarium act as secondary parasites and hasten destruction of the host.

5. Evidence is presented that *Phoma terrestris* is the cause of pink root of onions, not only in California but in other States as well, and that hosts other than Allium may be attacked.

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LENTINUS LEPIDEUS FR.: A CAUSE OF HEART ROT OF LIVING PINES

W. W. WAGENER

The scaly Lentinus, *Lentinus lepideus* Fr., is well-known as a saprophyte, decaying coniferous stumps, logs, foundation timbers, ties, etc., over a wide range in the North Temperate Zone.

As a heart rot of living trees it appears to be much less familiar. Hedgcock (3) in 1912 reported it or a closely related form as attacking the heartwood of both living and dead conifers in the National Forests but, owing to the scope of the paper, no details of his observations were included. Meinecke (6), in speaking of the sporophores of the fungus found on the butts of *Pinus jeffreyi* A. Murr., *P. ponderosa* Doug., and *P. contorta* Doug. in California, stated that occasionally they grew "on the dead roots of living trees and also high up on the trunk." A species of Lentinus parasitic on the roots of conifers in the western United States has been mentioned by Weir (8). He regarded it as a probably undescribed species but has verbally informed the writer that the fungus to which he referred is the one commonly known as *Lentinus lepideus* in the West. More recently, Martin (5, p. 407) observed sporophores of the fungus growing from branch scars on a living pine in Washington, D. C. While the occurrence of *Lentinus lepideus* or closely related forms in connection with living trees has thus been reported for some time, the fact seems not to have gained general attention and is not referred to in our pathological texts.

The situation is no doubt due in part to the uncertainty, as voiced by Hedgcock and Weir, regarding the true identity of the western fungus. While there appears to be some difference between the western form and the common one of the eastern United States and Europe, yet, as Harper (2) has pointed out, the species is everywhere quite variable and it is questionable whether the western fungus is any more entitled to specific rank than any one of the morphological variations found elsewhere. In the present paper common custom will be followed in referring to the fungus simply as *Lentinus lepideus* without entering the question as to the correctness of such usage.

The original report by Hedgcock was based mainly on field notes and collections made by him in connection with the Timber and Forest Disease Survey conducted for the United States Department of Agriculture some years ago. The fungus was noted at several places in the Western and Middle-western States with living *Pinus ponderosa*, *P. contorta*, and *P. banksiana* Lamb. as hosts. In some instances sporophores were found con-

nected with a brown heart rot within the tree, from which the fruiting bodies issued through knotholes or open wounds. In general he found that the rot appeared to be confined to the heartwood but in one lodgepole pine (*P. contorta*) the sapwood adjacent to a basal fire scar was also involved.

Collection and field notes in the files of the San Francisco Office of Forest Pathology contain a number of records of the fungus observed on living western yellow pine (*P. ponderosa*) and lodgepole pine by Meinecke. Sporophores were noted growing from the bases of trees, from roots near the base or directly from decayed heartwood through openings in the trunks.

FIELD OBSERVATIONS ON INDIVIDUAL CASES IN CALIFORNIA

Within the past few years additional finds of the fungus have been made in California on living western yellow pine and on sugar pine (*P. lambertiana* Doug.), a previously unrecorded host. Several of these cases were

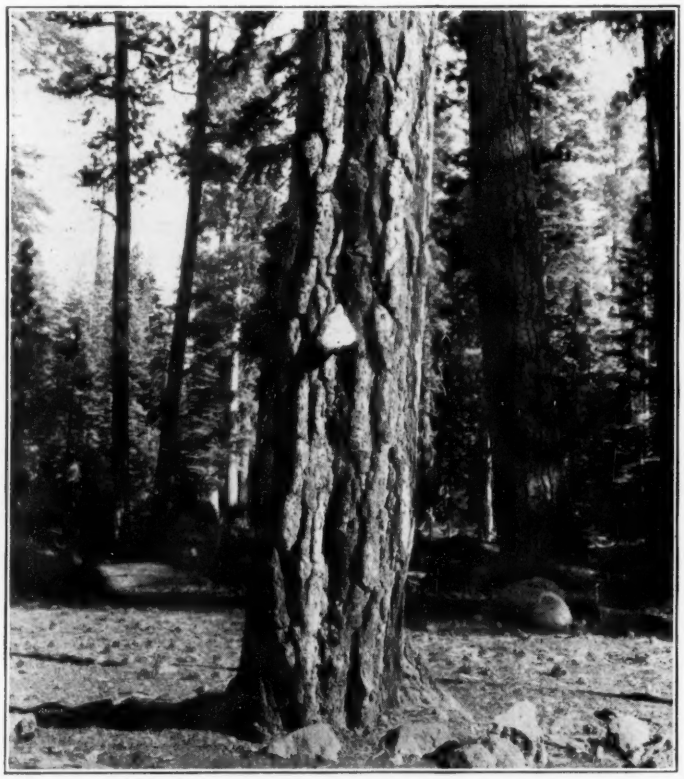

FIG. 1. Sporophore of *Lentinus lepideus* growing from an old, bark-closed wound on *Pinus ponderosa*, Westwood, California.

encountered in the course of a study of decay carried out under the direction of Dr. E. P. Meinecke in connection with the logging of a government timber sale in the central Sierra Nevada Mountains. The stand was of mixed conifers of large size, mostly mature, growing at an elevation of 5000 feet.

The first case noted occurred in a western yellow pine measuring 56 inches in diameter breast high and originally 180 feet tall. At the time of felling, early in September, only a standing snag 70 feet in height remained, the entire top having broken out during a severe windstorm the previous fall. The diameter of the bole at the break was about 28 inches. Western yellow pine is ordinarily very resistant to breakage of this sort and the fact that no similar damage was shown by adjacent trees pointed to some weakness of the bole in the region of the break.

The tree was examined about four weeks after felling. A dry, mature sporophore of *Lentinus lepideus* about 15 cm. in diameter was found projecting from a recess in the decayed wood on the top end of the snag. It arose from a felty, mycelial pad formed over the surface of the wood and was of the normal pileate type. Its shape and condition indicated that the sporophore had matured and hardened on top of the snag before felling.

The lower end of the long broken-off top which had lain on the ground over winter bore a sterile fructification consisting of a curving, branched, compound stipe with brown, conically pointed tips. It arose from a mycelial pad similar to that at the base of the normal sporophore, except for soil and litter embodied in the surface. The production of sterile fructifications of this type is known to be a common phenomenon for *Lentinus lepideus*. The shapes are frequently grotesque (7, p. 411). Experiments by Buller (1) showed that light has a strong influence in determining the form of the fruiting body. In the present case the normal sporophore developed in full sunlight while the end of the break bearing the abortive one, lay so that it was constantly, though not heavily, shaded. In view of Buller's experiments, this may account for the dissimilar development of the two fruiting bodies. Normal pileate sporophores were later produced from both portions of the down tree.

Decay and discoloration in connection with the sporophores extended roughly spindle-shape for 12 feet above and 8 feet below the break, or a total distance of 20 feet along the bole in both heartwood and sapwood. The rest of the tree was sound. The decay centered around a long, narrow wound, dating from 1895, on the side of the bole and probably due to a falling tree or snag. This wound quite evidently served as the entrance point for the fungus, its subsequent development weakening the bole so that it was unable to withstand the stress of the heavy wind.

An important factor in the weakening was the death of the callus-tissue and sapwood for about a foot on each side of the wound after normal callus formation had continued for a number of years. The dead area was found to be in various stages of decay from Lentinus and was covered for the greater part by a creamy-white mycelial felt about 1/16 of an inch thick, which had formed between the wood and the overlying bark. No definite cause could be ascribed for the death of this zone of sapwood. However, the presence of the fungus apparently throughout the decayed area and the absence of indications of any other cause for death suggested its possible connection with the killing.

The rot itself was not pronounced except in the vicinity of the wound where, for a depth of about 6 inches, the wood had disintegrated into long, rectangular, brown flakes or chunks of carbonaceous texture. The rest of the affected wood appeared solid in cross section, but, when tested with the point of a knife blade, proved to be softer than normal and lacking in elasticity. Thin mycelial felts showed in longitudinal section but were confined to the small shrinkage checks near the surface of the break. The color of the wood was a slightly darker yellow than normal, shading into a reddish tinge in places. The sporophores did not develop from the badly disintegrated outer wood but from the more solid heartwood about 10 inches in from the outside of the trunk.

A loss of 3000 board feet resulted from the decay and the breakage caused by it in this tree.

A second case of the rot was disclosed by the felling of a living yellow pine 40 inches in diameter and 188 years of age at stump height. The entire heartwood at the stump was involved in a spongy, reddish brown decay with a resinous, aromatic odor suggesting *Lentinus lepideus*. A cluster of two sporophores and an abortive stipe of the fungus were found hidden under a layer of needles and dry duff about 2 feet from the base of the tree. They arose from a shallow, decayed root about an inch in diameter. The decay appeared to connect through this root with that in the butt of the tree though conditions prevented excavation to determine this point definitely. Some support was afforded by the fact that agar cultures of the decayed wood from the stump gave typical *Lentinus lepideus* growths. The rot was confined to the butt of the tree, resulting in a deduction for cull of 140 board feet in the scale of the first log. Infection had presumably taken place through a fire scar, open since about 1850, at the base of the tree.

A third case of the disease was found in the upper bole of a sugar pine 67 inches in diameter breast high. The tree had been broken off at 133 feet from the ground, resulting in the loss of much of the crown but leaving a number of large live limbs just below the break. The diameter at the break

was about 14 inches, but it increased rapidly below this point. Decay extended down in the heartwood of the trunk for 11.5 feet, attaining a maximum diameter of 20 inches. It also entered the bases of several of the live branches. The center for some distance down had become hollow, and attached to the decay on the sides of the hollow were a number of stained and misshapen fruit bodies, mostly abortive, and some so covered with bits of decayed wood and débris as to be scarcely recognizable. One or two had developed sufficiently to be identified as *Lentinus lepideus*. The length of time the fungus had been present could not be determined. A cull loss of about 100 board feet resulted.

Several additional living yellow pines affected with the decay have since been noted by the writer. In one of 45 inches diameter, found later on the same sale area, the fungus had entered through an old broken top and had destroyed the heartwood for 40 feet below, resulting in a cull loss of 1060 board feet. The decayed portion of the bole split its entire length when the tree was felled.

Another tree, 48 inches in diameter, on which a Lentinus sporophore had been seen issuing from an exposed root, was examined. Some years before, a road had been excavated on the slope immediately below this tree, resulting in the soil and a number of large roots being cut away on the lower side. Other roots were partly exposed. Due to the location it was impossible to fell the tree for examination but ax cuts and soundings showed the base to be badly decayed for at least 8 feet above ground. On the side next to the road the sapwood was dead and had decayed until only a thin shell of firmer dry wood remained on the surface.

About a month intervened between the time the sporophore was noted and the time the examination was made. In the meantime the fruiting body had been removed by man or animal so completely that not even its point of attachment could be definitely determined.

The root from which it was thought to have issued was about 4 inches in diameter and was virtually exposed for about a foot of its length, beginning three feet out from the tree. A cut made through at the middle of the exposed length showed nearly two-thirds of the cross section decayed. The rest was still alive. The root was next excavated back to its junction with the base of the tree and opened up. It was seen that the decay continued back and connected with the main body of rot in the base of the trunk.

In order to make certain that the decay in the root and that in the trunk were identical, agar cultures were made of each. The growths obtained agreed in all particulars with each other and with authentic cultures of the fungus. This case supports previous indications that the decay may extend into the roots of a tree while the latter is still alive.

THE DECAY

Early stages of the decay in living pines have no very marked characteristics. The wood becomes brash and darkens slightly in color, with a faint reddish tinge appearing in some cases. Little or no shrinkage in excess of that in the normal wood takes place. Thin mycelial felts are formed in checks or shakes, particularly in portions from which sporophores will later develop.

In later stages the decayed wood is friable, crushing to a powder when rolled between the thumb and finger, but with a cheesy or sticky character when fresh due to contained moisture and to infiltration of oleoresins. These latter the fungus appears to be able to liberate in unusual degree. The infiltration is particularly noticeable in decay in the butts of trees or in the vicinity of fire scars or wounds and causes the decay to appear darker in color than when dried out.

The essential oils volatilize rather rapidly from freshly-exposed decay, giving it a highly aromatic, resiny odor, noticeable at some feet distant. A fragment no larger than one's little finger will, when freshly broken, sometimes scent an entire room. Coupled with the resiny odor is one of a fungous nature, the combination making an odor peculiar, so far as the writer knows, to the decay of *Lentinus lepideus* and readily recognized, once one has become acquainted with it.

Some shrinkage cracks form in fresh decay of the later stages but, as a rule, it is not until the decay has been exposed and subjected to drying that it breaks up into the brittle, cubical chunks typical of the carbonizing rots. In *Lentinus lepideus* the chunks are rather large and rectangular, corresponding in size to those of *Fomes laricis* (Jacq.) Murr. except in sapwood where smaller cubes are formed. A variation sometimes found in decayed heartwood is the breaking up into thin rectangular flakes, the wood separating tangentially along the annual rings. This is brought about by the greater resistance of the summerwood to the action of the fungus and is more likely to be found in intermediate stages of the decay. In color, dry decay varies from cinnamon buff and clay to cinnamon and sayal brown, weathering on exposure to a dark brown or grayish brown. Thin, tough felts of mycelium are ordinarily present.

The dry decay sometimes resembles that of *Fomes laricis* so closely that specimens of each placed side by side can not be told apart visually though a distinction can easily be made on the basis of odor. The microscopic characters of the two decays are very similar (4). Old weathered decay is almost indistinguishable from that of *Polyporus schweinitzii* Fr. In a fresh state the decay, because of its odor, absence of checking into cubical form, and its consistency, can scarcely be confused with either of the others mentioned.

As indicated by the field cases reported, the rot is not characteristically limited to any particular portion of the bole. However, owing to the concentration around the base of the tree of wounds favorable to its entrance, infections of the fungus are more likely to be found in the heartwood of the butt.

HOSTS AND DISTRIBUTION

A summary from records at hand shows the following distribution for observed cases of the fungus on living trees:

California	on	*Pinus*	*ponderosa*	7	localities.
"	"	"	*contorta*	2	"
"	"	"	*lambertiana*	1	locality.
Arizona	"	"	*ponderosa*	1	"
Montana	"	"	*contorta*	1	"
Minnesota	"	"	*banksiana*	1	"
Dist. of Columbia	"	"	sp.	1	"

For several of the Californian localities and also for the one in Arizona more than one case of the rot has been found.

These distributional records and supporting field observations indicate that decay from *Lentinus lepideus* in living pines is not uncommon in the west Sierran region of California but to be found only occasionally or rarely elsewhere in the country. The Californian localities are well distributed over the west slope of the Sierra Nevada range.

The reasons for the apparent greater prevalence in California are not entirely clear. It may be noted in this connection however that the fungus is an extremely common saprophyte on stumps and down logs in the region and that the yellow-pine stands have a heavy representation of large, mature trees, most of which bear fire scars or wounds of some sort.

SUMMARY

Lentinus lepideus, well-known as a saprophyte, also causes a heart rot of living pines, particularly of the western yellow pine, *P. ponderosa*. It also has been found in *Pinus contorta*, *P. lambertiana*, and *P. banksiana*.

The fungus enters through wounds and may attack any part of the heartwood of the bole or extend into the roots. In rare cases the sapwood is also affected.

A friable decay, often characterized by an infiltration of oleoresins, is caused. The dry decay resembles that of *Fomes laricis* but may be distinguished by the resiny, fungous odor.

Field observations and collection records indicate that the decay is fairly common in living pines in the forests of the Sierra Nevada in California and rare elsewhere in the United States.

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THE USE OF ACIDULATED MERCURIC CHLORIDE IN DISINFECTING POTATO TUBERS FOR THE CONTROL OF RHIZOCTONIA[1]

J. G. Leach, H. W. Johnson, and H. E. Parson[2]

Experimentation for many years by numerous workers throughout the United States has demonstrated conclusively that seed-tuber disinfection is an effective means of reducing losses from scab, blackleg, and rhizoctonosis. Tuber disinfection has never given perfect control of these diseases because none of them is exclusively tuber-borne. Nevertheless, under most conditions, thorough tuber disinfection has greatly reduced the amount of infection.

Since the early work of Bolley (2) and Arthur (1) many different disinfectants have been used and many different methods of application have been recommended. Some of these have been very effective, while others have been less valuable. Perhaps the most successful disinfectants have been formaldehyde and mercuric chloride. These have proved very effective in countless experiments, and for many years have been used as standards of comparison.

In determining the value of a method of tuber disinfection there are two factors of major importance to consider: First, the effectiveness of the treatment in disinfecting the tubers, and second, the practicability of the method in the hands of the farmer.

The lethal action of a disinfectant on the sclerotia of Rhizoctonia generally has been considered as the limiting factor in determining its effectiveness, for any disinfectant which penetrates and kills the sclerotia doubtless would destroy the pathogenes causing blackleg and scab.

In judging the practicability of the method in the hands of the farmers one must consider such factors as the probability of injury to the seed tubers, the cost of materials, the availability of necessary facilities for proper application, and the time and labor involved. Experience has shown that unless these factors are favorable the method is not likely to be used generally, regardless of its merits as an effective disinfectant.

The principal objections of potato growers to the use of tuber disinfection have been the time, trouble, and labor involved. For many years, the long-soak formaldehyde and mercuric-chloride methods were the stand-

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[2] The authors wish to express their appreciation to Mr. T. M. McCall, Horticulturist of the Northwest School and Station, Crookston, for his splendid cooperation throughout this work and for his care of the experimental plots at Crookston.

ard methods recommended by most experiment stations. These methods were practicable when only a few bushels were to be disinfected, but the time, labor, and equipment were almost prohibitive when tubers for large acreages were to be treated.

The first successful effort to remedy these difficulties was made in 1919 by Melhus and Gilman (5) in devising the hot-formaldehyde method. The hot-mercuric-chloride method also has been used successfully in some States. The virtue in the use of hot solutions lies in their more rapid penetration and killing of the Rhizoctonia sclerotia and other tuber-borne pathogenes. It has been found that equally good or better disinfection could be obtained in three minutes at 49° C. than in two hours by the old two-hour soak in the cold solution. The use of hot solutions reduced the time and labor required and has been much more practicable than the old two-hour cold-soak method when relatively large quantities of tubers are involved.

The method has, however, introduced other difficulties, the most important of which are (a) the necessity of more complicated apparatus for heating the solution, (b) the difficulty of accurately controlling the temperature, (c) the danger of injury to the tubers if the solution is allowed to become a few degrees too hot, or (d) incomplete disinfection if the solution is allowed to become too cool.

Realizing these difficulties, an effort has been made to devise a method of disinfection which would eliminate the disadvantages but retain the advantages of the short hot treatment. While planning these experiments the attention of the writers was called to the trials of Cunningham with acidulated mercuric chloride. Cunningham in 1925 (3) found in a series of laboratory experiments that the addition of a small amount of hydrochloric acid to the mercuric chloride solution greatly increased its effectiveness in killing the sclerotia of *Rhizoctonia solani* Kühn. Among other things, he demonstrated that if 1 per cent of hydrochloric acid was added to a 1–1000 solution of mercuric chloride complete killing of the sclerotia resulted when infected tubers were soaked for only five minutes and then covered with wet sacks over night. In the following year, Cunningham and Neill (4) presented data from a field test based on the previously reported laboratory tests. From these data they concluded that the method, "though giving excellent results in the laboratory, failed to control Corticium disease in the field."

Although the data presented did not show consistent increases in yield from disinfected tubers as compared with the controls, they did show a fairly consistent and decided reduction in the number of sclerotia developing on tubers produced by disinfected seed pieces. Inasmuch as the production of uniformly high-grade tubers as well as clean tubers for seed purposes is frequently as important as or more important in the United

States than increased yields, it appeared that the method gave more promise than the conclusions of Cunningham and Neill would indicate.

The acidulated-mercuric-chloride method, in various combinations, has been tested in comparison with other methods for three years in Minnesota. The results obtained appear sufficiently promising to justify its recommendation for more extensive trial. The data for the three years are presented in this paper.

MATERIALS AND METHODS

The trials were made in two different parts of the State. One set of trials was made at University Farm, St. Paul, and the other at the Northwest School and Station at Crookston, Minnesota. In general, the rhizoctonosis is somewhat more prevalent at the latter place and soil infestation is more common.

Tubers of the Early Ohio variety bearing Rhizoctonia sclerotia were used in all experiments. Each year some of the tubers were disinfected before cutting and some after cutting, but, as no appreciable differences were obtained, the results have been considered together. In all trials, except those at University Farm in 1926 and 1927, each plot consisted of 50 seed pieces planted 14 inches apart in rows 3 feet apart. At University Farm, in 1926, the plots consisted of only 24 plants. For data on tuber infection the number of plots for each treatment were as follows:

St. Paul, 1926, three; 1927, five; and 1928, four.

Crookston, 1926, three; 1927, four; and 1928, seven.

The data, therefore, are based on approximately 222 plants for each treatment in 1926, 450 in 1927, and 550 in 1928.

In 1927, the St. Paul plots were so badly washed by heavy rains shortly after planting that a very irregular stand resulted. These plots were therefore not used for the yield data. The data on stem lesions are based on one plot from each treatment in each place in 1926 and 1927 and from two plots in each place in 1928, a total of eight plots for each treatment.

The treatments, which differed somewhat each year, were as follows:

Key to 1926 Experiments

1. Standard mercuric chloride (1–1000), 2 hour soak, cold.
2. Mercuric chloride (1–500), 5 minute soak, cold.
3. Mercuric chloride (1–1000) + 1 per cent HCl, 5 minute soak, cold.
4. Mercuric chloride (1–1000) + 2 per cent HCl, 5 minute soak, cold.
5. Mercuric chloride (1–500) + 1 per cent HCl, 5 minute soak, cold.
6. Mercuric chloride (1–500) + 2 per cent HCl, 5 minute soak, cold.
7. Check. No treatment.
8. Semesan, 0.25 per cent, 5 minute soak, cold.
9. Semesan, 0.25 per cent + 2 per cent HCl, 5 minute soak, cold.
10. Hot formaldehyde (1–120), 3 minute soak, 49° C.

Key to 1927 Experiments

1. Standard mercuric chloride (1–1000), 2 hour soak, cold.
2. Mercuric chloride (1–1000), 5 minute soak, cold.
3. Hot mercuric chloride (1–1000), 2 minute soak, 49° C.
4. Mercuric chloride (1–1000) + 1 per cent HCl, 5 minute soak, cold.
5. Mercuric chloride (1–500) + 1 per cent HCl, 5 minute soak, cold.
6. Check A. No treatment.
7. Mercuric chloride (1–500) + 1 per cent HCl, 10 minute soak, cold.
8. Mercuric chloride (1–500) + 1 per cent HCl, 20 minute soak, cold.
9. Mercuric chloride (1–500) + 1 per cent HCl, 40 minute soak, cold.
10. Hot formaldehyde (1–120), 3 minute soak, 49° C.
11. Formaldehyde (1–120), 5 minute soak, cold.
12. Formaldehyde (1–120) + 1 per cent HCl, 5 minute soak, cold.
13. Mercuric chloride (1–1000) + 0.2 per cent citric acid, 5 minute soak, cold.
14. Mercuric chloride (1–1000), sat. solution of picric acid, 5 minute soak, cold.
15. Saturated solution of picric acid in water, 5 minute soak, cold.
16. Check B. No treatment.
17. Semesan Bel, No. 2, 1 lb. to 10 pints water, instantaneous dip, cold.
18. Semesan Bel, No. 21, 1 lb. to 15 pints water, instantaneous dip, cold.
19. Bayer Dipdust, 1 lb. to 20 pints water, instantaneous dip, cold.
20. Bayer Special dust, No. 181, 1 lb. to 5 gallons water, instantaneous dip, cold.

Key to 1928 Experiments

1. Standard mercuric chloride (1–1000), 2 hour soak, cold.
2. Hot mercuric chloride (1–1000), 2 minute soak, 49° C.
3. Mercuric chloride (1–1000) + 1 per cent HCl, 5 minute soak, cold.
4. Check A. No treatment.
5. Mercuric chloride (1–1000) + 1 per cent HCl, 20 minute soak, cold.
6. Mercuric chloride (1–1000) + 0.2 per cent citric acid, 5 minute soak, cold.
7. Mercuric chloride (1–500) + 1 per cent HCl, 5 minute soak, cold.
8. Check B. No treatment.
9. Hot formaldehyde (1–120), 3 minute soak, 49° C.
10. Formaldehyde (1–120) + 1 per cent HCl, 5 minute soak, cold.
11. New Improved Semesan Bel, 1 lb. to 20 pints water, instantaneous dip, cold.
12. Bayer Dipdust, 1 lb. to 20 pints water, instantaneous dip, cold.

Local tap water, which was relatively hard, was used in all experiments. The temperature of the cold solutions was approximately 10° C. Where hydrochloric acid was added to the solution, the percentage is figured in terms of volume of commercial hydrochloric acid of approximately 31 per cent strength. Thus "1 per cent HCl" means one c.c. of commercial hydrochloric acid to each 100 c.c. of the disinfecting solution.

The notes on stem lesions were taken when the plants were about 12 inches high. The plants were pulled out of the ground and washed, and the stems were examined for lesions. In the figures given, no distinction is made between light and heavy infections. If proper values had been given to different degrees of infection, the differences would have been more striking.

Notes on tuber infection and yield were taken at harvest. The tubers were thoroughly washed and those having even a few small sclerotia were considered infected. Here, also, the differences would have been more striking if consideration had been given to the different degrees of infection and to the grading qualities of the different lots.

DISCUSSION OF RESULTS

The averages of each treatment for each year are presented in graphs (Figs. 1–5) by means of which the relative value of the treatments may be observed.

From a study of the graphs it is obvious that, of those treatments which have been given sufficient trial, the standard mercuric-chloride method, the acidulated-mercuric-chloride method, and the hot-formaldehyde method gave better results than the other treatments. It is also quite obvious that none of the organic mercury compounds reduced the amount of the disease sufficiently to justify their consideration for practical use. In fact, in many

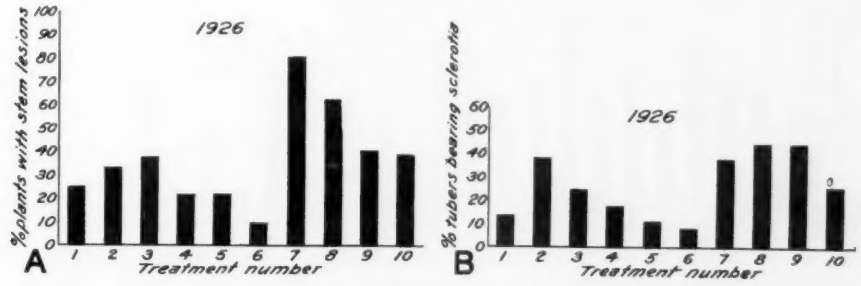

FIG. 1. A. Results of seed-tuber disinfection in 1926 in terms of average percentage of plants with stem lesions, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1926 in terms of average percentage by weight of tubers bearing sclerotia, St. Paul and Crookston.

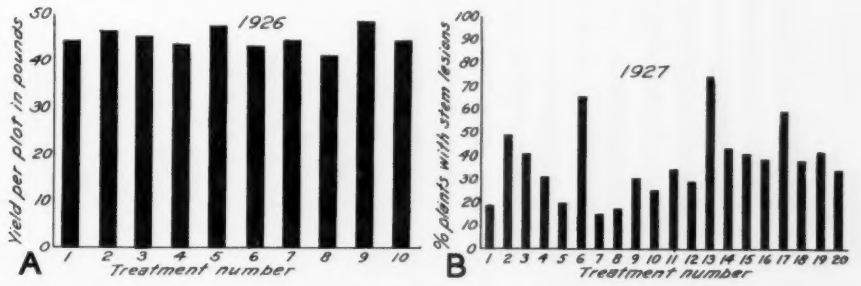

FIG. 2. A. Results of seed-tuber disinfection in 1926 in terms of average yield in pounds per plot, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1927 in terms of average percentage of plants with stem lesions, St. Paul and Crookston.

cases the yield from treated tubers was almost as badly diseased as that from the checks.

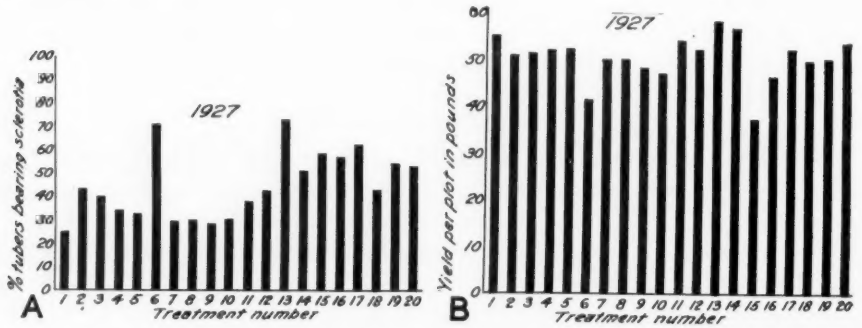

FIG. 3. A. Results of seed-tuber disinfection in 1927 in terms of average percentage by weight of tubers bearing sclerotia, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1927 in terms of average yield in pounds per plot, Crookston.

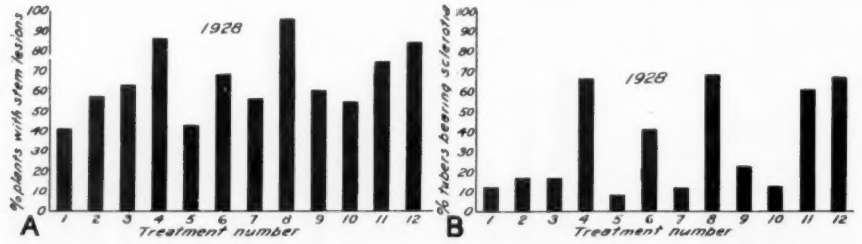

FIG. 4. A. Results of seed-tuber disinfection in 1928 in terms of average percentage of plants with stem lesions, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1928 in terms of average percentage by weight of tubers bearing sclerotia, St. Paul and Crookston.

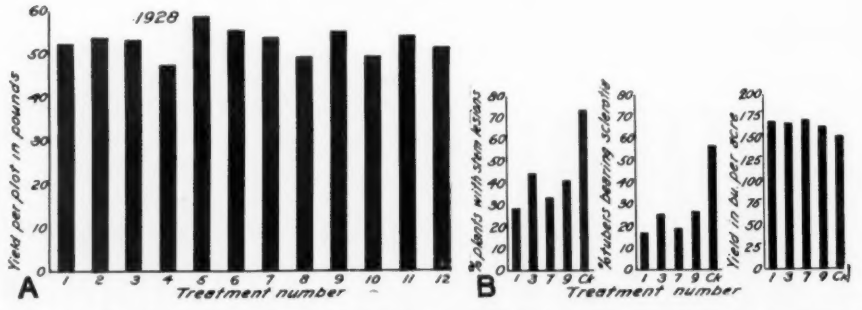

FIG. 5. A. Results of seed-tuber disinfection in 1928 in terms of average yield in pounds per plot, St. Paul and Crookston. B. Results of seed-tuber disinfection. Three-year average for the standard mercuric chloride treatment (1), acidulated mercuric chloride (1–1,000) + 1% HCl, (3), acidulated mercuric chloride (1–500) + 1% HCl, (7), the hot formaldehyde treatment (9), and the check (ck).

TABLE 1.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7),*

TABLE 1.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7), compared with the results obtained with the standard mercuric-chloride method (1), the hot formaldehyde method (9), and the check (ck), in terms of percentage of tubers bearing sclerotia of Rhizoctonia solani*

Year and place	Treatment[a]	Percentage Rhizoctonia	Comparison with check		Comparison with (7)	
			Difference	Diff./P.E.	Difference	Diff./P.E.
St. Paul, 1926	1	9.00 ± 0.64	−26.03 ± 3.46	7.52	− 5.00 ± 2.47	2.02
	7	14.00 ± 2.39	−21.03 ± 4.12	5.07		
	9	28.23 ± 0.41	− 6.80 ± 3.46	2.00	+14.23 ± 2.42	5.88
	ck.	35.03 ± 3.43				
Crookston, 1926	1	19.03 ± 3.48	−24.34 ± 4.36	5.57	+ 9.03 ± 3.78	2.38
	7	10.00 ± 1.50	−33.37 ± 3.04	10.97		
	9	23.90 ± 1.60	−19.47 ± 3.09	6.30	+13.90 ± 2.19	6.34
	ck.	43.37 ± 2.65				
St. Paul, 1927	1	5.80 ± 0.51	−43.56 ± 1.21	36.00	− 7.56 ± 2.16	3.48
	7	13.36 ± 1.45	−36.00 ± 1.81	19.89		
	9	14.30 ± 0.69	−35.06 ± 1.30	26.96	+ 0.94 ± 1.60	0.58
	ck.	49.36 ± 1.10				
Crookston, 1927	1	43.77 ± 3.82	−34.85 ± 4.36	7.99	− 8.68 ± 5.61	1.54
	7	52.45 ± 4.12	−26.17 ± 4.58	5.71		
	9	47.60 ± 6.90	−31.02 ± 7.19	4.31	− 4.85 ± 8.03	0.60
	ck.	78.62 ± 2.03				
St. Paul, 1928	1	15.72 ± 1.67	−81.26 ± 1.75	46.43	− 1.73 ± 2.13	0.81
	7	17.45 ± 1.33	−79.53 ± 1.43	55.61		
	9	31.67 ± 2.36	−65.31 ± 2.41	27.09	+14.22 ± 2.88	4.94
	ck.	96.98 ± 0.54				
Crookston, 1928	1	10.26 ± 1.30	−26.39 ± 1.62	16.28	+ 3.66 ± 1.36	2.69
	7	6.58 ± 0.42	−30.07 ± 1.05	28.63		
	9	14.08 ± 0.93	−22.57 ± 1.34	16.83	+ 7.50 ± 1.01	7.42
	ck.	36.65 ± 0.97				
Three year average, St. Paul and Crookston. Total of 26 plots	1	16.26 ± 0.48	−39.17 ± 2.10	18.65	− 1.60 ± 0.69	2.32
	7	17.86 ± 0.50	−37.57 ± 2.11	17.80		
	9	24.75 ± 1.66	−30.68 ± 2.63	11.66	+ 6.89 ± 1.73	3.98
	ck.	55.43 ± 2.05				

[a] The numbers used for these treatments were not uniform for the three years. The numbers used in the tables are those used in 1928.

TABLE 2.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7), compared with results obtained with the standard mercuric-chloride method (1), the hot-formaldehyde method (9), and the check (ck), in terms of total yield in pounds per plot*

Year and place	Treatment[a]	Yield	Comparison with check		Comparison with (7)	
			Difference	Diff./P.E.	Difference	Diff./P.E.
St. Paul, 1926	1	19.33 ± 0.19	+ 4.58 ± 0.34	13.47	+ 0.41 ± 0.22	1.86
	7	18.92 ± 0.12	+ 4.17 ± 0.30	13.90		
	9	17.25 ± 0.39	+ 2.50 ± 0.48	5.20	− 1.67 ± 0.40	4.17
	ck.	14.75 ± 0.28				
Crookston, 1926	1	47.13 ± 1.08	− 5.37 ± 1.92	2.80	− 5.20 ± 1.57	3.31
	7	52.33 ± 0.90	− 0.17 ± 1.68	0.10		
	9	50.00 ± 0.18	− 2.50 ± 1.53	1.63	− 2.33 ± 0.92	2.53
	ck.	52.50 ± 1.43				
Crookston, 1927	1	55.37 ± 1.03	+ 11.00 ± 1.56	7.05	+ 2.66 ± 1.07	2.48
	7	52.69 ± 0.33	+ 8.32 ± 1.21	6.88		
	9	49.69 ± 2.22	+ 3.32 ± 2.51	1.32	− 5.00 ± 2.24	1.56
	ck.	44.37 ± 1.17				
St. Paul, 1928	1	48.75 ± 0.86	+ 2.50 ± 1.02	2.45	− 2.94 ± 0.92	3.19
	7	51.69 ± 0.32	+ 5.44 ± 0.63	8.63		
	9	52.31 ± 1.58	+ 6.06 ± 1.67	3.62	+ 0.62 ± 1.61	0.38
	ck.	46.25 ± 0.55				
Crookston, 1928	1	55.43 ± 0.92	+ 5.18 ± 1.07	4.84	− 0.07 ± 1.10	0.06
	7	55.50 ± 0.61	+ 5.25 ± 0.81	6.48		
	9	57.71 ± 0.67	+ 7.46 ± 0.86	8.51	+ 2.21 ± 0.90	2.45
	ck.	50.25 ± 0.54				
Three year average, St. Paul and Crookston. Total of 21 plots*	1	50.57 ± 0.29	+ 4.80 ± 0.41	11.72	− 0.68 ± 0.37	1.84
	7	51.25 ± 0.24	+ 5.48 ± 0.39	11.50		
	9	50.35 ± 0.35	+ 4.58 ± 0.45	10.18	− 0.90 ± 0.42	2.14
	ck.	45.77 ± 0.30				

* Due to heavy, washing rains shortly after planting, the stands at St. Paul in 1927 were so irregular that the yield data were not reliable. The yield data for St. Paul in 1927 are therefore not included here.

[a] The numbers used for these treatments were not uniform for the three years. The numbers used in the tables are those used in 1928.

TABLE 3.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7), compared with results obtained with the standard mercuric-chloride method (1), the hot-formaldehyde method (9), and the check (ck), in terms of percentage of plants with stem lesions*

Date and place	Treatment[a]	Percentage of plants with stem lesions	Comparison with check		Comparison with (7)	
			Difference	Diff./P.E.	Difference	Diff./P.E.
Average of three years at St. Paul and Crookston. Total of 8 plots	1	32.00 ± 1.60	−47.07 ± 2.25	20.92	−6.77 ± 2.62	2.58
	7	38.77 ± 2.08	−40.30 ± 2.61	15.44	———	———
	9	46.26 ± 2.40	−32.81 ± 2.87	11.43	+7.49 ± 3.17	2.36
	ck.	79.07 ± 1.58	———	———	———	———

[a] The numbers used for these treatments were not uniform for the three years.
The numbers in the tables are those used in 1928.

In 1926, several strengths of acidulated mercuric chloride were used. Of these, the one containing 2 per cent hydrochloric acid gave the best control, but it was not enough better than the one containing 1 per cent hydrochloric acid to justify the additional cost of the material. Therefore, all subsequent experiments were made on the basis of 1 per cent-hydrochloric-acid content with different amounts of mercuric chloride and different lengths of soaking. Considering the results of the three-year period, the solution containing 1 per cent hydrochloric acid and mercuric chloride at the rate of 1–500 appears to be the most effective.

In order to evaluate more accurately the relative effectiveness of this method, the results obtained with it have been compared biometrically with those of the standard mercuric-chloride method, the hot-formaldehyde method, and the check. These comparisons are given in tables 1 to 3.

From the data presented in the graphs and the table, it may be concluded that:

1. The mercuric-chloride treatments gave better results than the hot-formaldehyde treatment.
2. The standard mercuric-chloride method resulted in slightly better control of Rhizoctonia than the acidulated mercuric-chloride method, although the difference is not strikingly significant.
3. The acidulated mercuric-chloride method resulted in yields equal to those of the standard mercuric-chloride method and slightly better than those of the hot-formaldehyde method.
4. All three of these treatments resulted in marked disease control and significant increases in yield as compared to the checks.
5. Considering the many advantages of the acidulated-mercuric-chloride method over the standard mercuric-chloride method or the hot-formaldehyde method, its performance is sufficient to justify more extensive trial under varied field conditions.

Advantages of the acidulated-mercuric-chloride method. As pointed out above, one of the greatest obstacles in the way of general use of seed-tuber disinfection is the time and trouble involved. In this respect the acidulated-mercuric-chloride method has many advantages over any method previously devised. These may be mentioned briefly as follows:

1. A soak of 5 minutes is sufficient to produce results practically equal to those of a two-hour soak with the standard mercuric-chloride method.
2. The use of hard water does not alter the effectiveness of the solution since the use of acid overcomes this.

3. Mercuric chloride dissolves very rapidly in the acidulated solution, while it dissolves very slowly and with difficulty in cold water.
4. No heating equipment is required.
5. No thermometer for temperature control is necessary.
6. There is little danger of seed injury by prolonging the time of soaking. No injury was produced by soaking as long as 40 minutes.[3]
7. With this latitude in time of soaking there is less danger of failure to control by too short exposure.
8. There is no danger of poor control by allowing the temperature of the solution to drop a few degrees.

The loss of strength of the acidulated solution. The rate of loss of acid and mercuric chloride from the solution determines the quantity of tubers which can be treated before the solution must be discarded or more acid and mercuric chloride added. In order to determine this rate of loss a solution was prepared in which seven successive lots of tubers were treated. The tubers were treated in an earthenware crock, in which just enough solution was placed to completely cover the tubers. The tubers were moderately dirty and were considered as an average seed lot. A sample of the solution was taken after each five-minute treatment. These samples were analyzed for acid and mercuric chloride content by the Division of Agricultural Biochemistry. The results are given in table 4. It is obvious

TABLE 4.—*The rate of loss of mercuric chloride and hydrochloric acid from the treating solution during the process of treating seven successive lots of tubers*

	Content of $HgCl_2$ in per cent	Content of HCl in per cent
Original solution	0.2266	0.376
After 1 treatment	0.2152	0.372
After 2 treatments	0.1997	0.353
After 3 treatments	0.2088	0.351
After 4 treatments	0.2047	0.347
After 5 treatments	0.2010	0.342
After 6 treatments	0.1974	0.338
After 7 treatments	0.1864	0.328

[3] Some seed injury has been observed in experiments made in 1929 where large quantities of tubers were treated and stored in large bins for several days before planting. Injury has also been observed where the treated tubers were planted in very dry soil. Injury in the latter case was not peculiar to the acidulated mercuric-chloride method, since every treatment used, including various modifications of the mercuric chloride and formaldehyde methods, resulted in some seed injury and delayed germination. Efforts are being made to find a means of avoiding this injury before the method is recommended for general use.

from these figures that at least eight and probably more lots of tubers could be treated in such a solution before it would be necessary to add more acid or mercuric chloride. More experiments are planned to check these results under field conditions. Such trials will be necessary also for devising the most convenient and practical procedure in applying the method.

SUMMARY

1. A solution of mercuric chloride (1–500) acidulated by the addition of 1 per cent by volume of commercial hydrochloric acid has been tested as a seed-tuber disinfectant in comparison with the standard mercuric-chloride method, the hot-formaldehyde method, and several other methods.

2. The experiments were performed at two different places in Minnesota and include data for three seasons. The data show that by using the acidulated solution as a five-minute cold soak better results are obtained than those obtained by the hot-formaldehyde method and virtually as good as those secured by the standard mercuric-chloride two-hour soak.

3. Experiments show that after treating seven successive lots of moderately dirty tubers in the acidulated solution only 17.7 per cent of the mercuric chloride and 12.2 per cent of the acid were lost.

4. The simplicity and ease of application of the acidulated mercuric-chloride method give the method many advantages over methods now in use and justify its recommendation for extensive trial under field conditions. Further experiments with the view of perfecting the method are under way.

5. Several of the organic-mercury treatments have been tested and all were found to be ineffective in reducing the amount of infection by *Rhizoctonia solani*.

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A BUTT-ROT OF BALSAM FIR CAUSED BY POLYPORUS BALSAMEUS PK.

ERNEST E. HUBERT

THE DISEASE

An interesting brown butt-rot of the balsam fir, *Abies balsamea* (L.) Mill., has been reported by Faull (1, 2) as causing serious damage in the pulpwood stands of Canada. It has also been found by the writer in the balsam-fir stands (4) of northern Wisconsin and Minnesota where its rapid development following the death of the host due to the spruce bud worm, *Cacoecia fumiferana* Clem., has largely been responsible for the fact that salvaging of such a stand after the third year is very nearly hopeless. The fungus causing this disease was noted by Peck (5, p. 46) as early as 1878 in the balsam stands of the Adirondack Mountains. Peck (6, p. 91) also collected a very similar fungus on *Tsuga canadensis* (L.) Carr., in New York, and named it *Polyporus crispellus* Pk.

The brown cubical rot, found in the butt and roots of balsam fir, is caused by *Polyporus balsameus* Pk. Three other fungi, *P. floriformis* Quel., *P. tephroleucus* Fr., and perhaps *P. crispellus* Pk., may be regarded as synonyms of the above fungus.

In 1922 the writer collected *P. balsameus* on living but suppressed trees of *Thuja occidentalis* L. in the Door-County region of Wisconsin. The butt-rot in these trees was similar to that observed in the balsam firs and no other fungus sporophore was found associated with these infections. Faull reported, in a letter to the writer, his having collected this fungus on the above host under conditions which he believed to indicate parasitism. In the Door-County cedars no evidence was obtained to indicate that the fungus was attacking the live sapwood but it was observed that the brown rot filled the entire heartwood area and left but a narrow margin of sapwood apparently unaffected. Like certain other wound fungi, under favorable conditions, *P. balsameus* may invade the sapwood zone.

SYMPTOMS

The external symptoms indicating the presence of this destructive butt-rot within the living tree are not very numerous. Whenever present, the typical sporophore of the fungus attached to roots or butt sections, is good evidence of butt-rot. At times, however, the fruiting bodies are not attached to the trunk or to exposed roots but develop on the ground at a point away from the tree where a root comes very near the surface. Under such conditions the sporophore more nearly fits the description given for

Polyporus floriformis and is characterized by a rosette-like development. This characteristic presence of fruiting bodies is in evidence only for a short period during the growing season, for insects destroy the sporophores soon after they become mature.

Basal fire scars, frost cracks, wind cracks, pin knots on lower trunk, and injuries to exposed roots are common exterior signs which indicate ease of infection by this fungus. Basal fire scars and frost cracks were nearly always noted in connection with trees showing balsam butt-rot.

Resin flow in the root crotches and along the exposed portions of roots is quite common and also serves as an excellent indicator of this rot, along with a general swollen appearance at the base of some of the trees. The

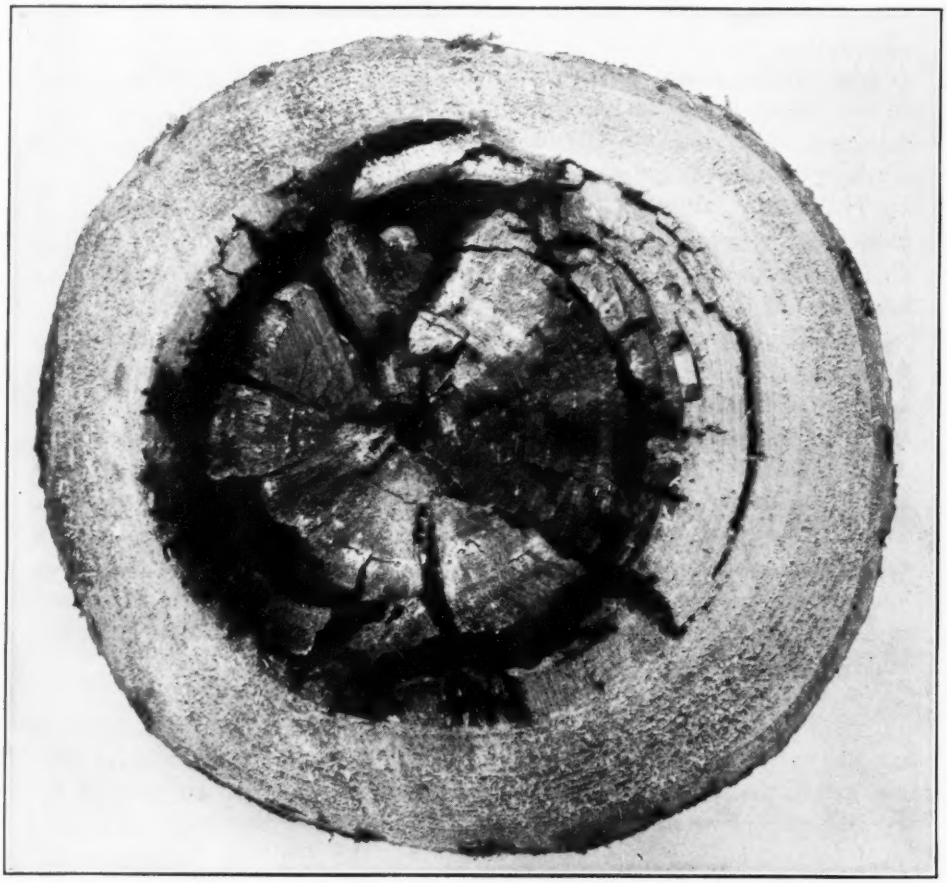

FIG. 1. The late stage of decay in *Abies balsamea* caused by *Polyporus balsameus*. This section was taken from a 55-year-old wind-thrown tree showing severely rotted roots. Note the shrinkage cracks.

typical rot in the late stage of its development, showing a downy layer of mycelium on the surfaces facing the shrinkage cracks, serves as an additional symptom, as does also the hollow sound at the butt section when the trunk is struck with an ax.

Swollen and punk knots have not been found associated with this disease.

DESCRIPTION OF THE ROT

Butt-rot of the balsam is classified under the brown-rots and is a characteristic brown, cubical rot (Fig. 1). The description of its early and late stages and the pathological histology of the infected wood is given below. The characteristics of the rot in *Thuja occidentalis* are very similar to those of *Abies balsamea*.

Early Stage. In longitudinal section, the early rot area is a warm buff, buff-yellow to light buff, fading into the whitish color of the normal wood. The wood in this stage is already somewhat softer than the noninfected wood. In this discolored area of early decay numerous hyphae are found extending into the apparently sound and normal-color wood beyond (4). In this stage the infected tissues show considerable evidence of fungous action. Some change in the cellular structure is evidenced by the use of the Bismark-brown-methyl-violet stain. Where this stain enters the cell walls they appear to be a deeper color than in the sound wood beyond. These infected tissues show numerous bore holes (Fig. 2) in the cell walls

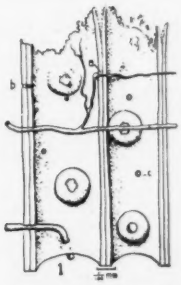

FIG. 2. Hyphae of *P. balsameus* in the wood cells of *Abies balsamea*. At a, a young thread has penetrated the wall. Bore holes produced by the fungus are shown at b and c. The decomposition of the cell wall and the corrosion and cracking of the bordered pits are prominent.

and a distinct corrosion of the surfaces of the cell wall. The cell walls are also reduced in thickness in comparison with the thickness of normal cell walls.

Late Stage. In end fracture, the color of this stage of decay is a sayal brown and when the rot is crushed to a powder it becomes a clay color. The older rot at the base of infected trees sometimes has a hazel-brown or snuff-

brown color. The cubes formed are large, the majority of the larger cracks running parallel to radial lines. The shrinkage cracks are of three types: (1) Those following annual rings, (2) those following radial lines, and (3) those in a plane traversing the stem. Within these shrinkage cracks is often formed a downy to powdery layer of white mycelium. The rotted wood crushes easily to a fine powder which feels soft and smooth between the fingers. Neither stage of decay shows zone lines.

Microscopic examination of the tissues in this stage of decay shows a very marked decomposition of the cell walls. They appear thinner, more corrosion is noted, and the bordered pits in the tracheid cells show irregular, enlarged and cracked pit openings (Fig. 2). Bore holes are numerous (Fig. 2, b, c) and the cell walls tear easily under sectioning and show ragged edges. The individual walls of the cells separate from each other in the heavily infected tissues, indicating a weakening or dissolution of the middle lamella. The reaction given when the ordinary reagents used to detect lingocellulose are applied indicates that the compound usually giving a cellulose reaction no longer responds. Apparently the cell substance remaining is composed principally of a lignin compound or has been so changed that the reaction is no longer characteristic. The hyphae are somewhat constricted where they pass through the cell walls. (Fig. 2, a.)

THE CAUSAL FUNGUS

When Koch's rules are applied to prove that *Polyporus balsameus* causes the characteristic butt-rot with which it is constantly associated, it is found that all these rules are satisfied. The fungus has been constantly found in direct association with the brown, cubical rot in the roots and basal trunks of conifers. A fungus has been repeatedly isolated from the infected wood, grown in pure culture, and inoculated under laboratory conditions on sterilized pieces of Sitka spruce wood. The fungus has produced in these pieces the rot symptoms characteristic of those of *P. balsameus*. It was later reisolated from the infected sticks and compared with the original pure cultures and with cultures obtained from the sporophores. The isolation cultures also were compared with the cultural characters for *P. balsameus* given by Miss Fritz (3).

TAXONOMY OF THE FUNGUS

Polyporus balsameus (Fig. 3) has not been collected extensively. J. R. Weir, who identified some of the early collections made in Wisconsin, probably has the largest collection of specimens from various regions yet assembled by any forest mycologist. The writer's collections are limited to sporophores and infected wood gathered at various points in northern Wisconsin and Minnesota. The sporophores appear in late August and early

September and soon disappear. This short period of fructification accounts, no doubt, for the seeming rarity of this plant. However, the sporophores are very abundant during the period of their production and vanish rapidly soon after maturity because of the attack of numerous grubs and beetles,

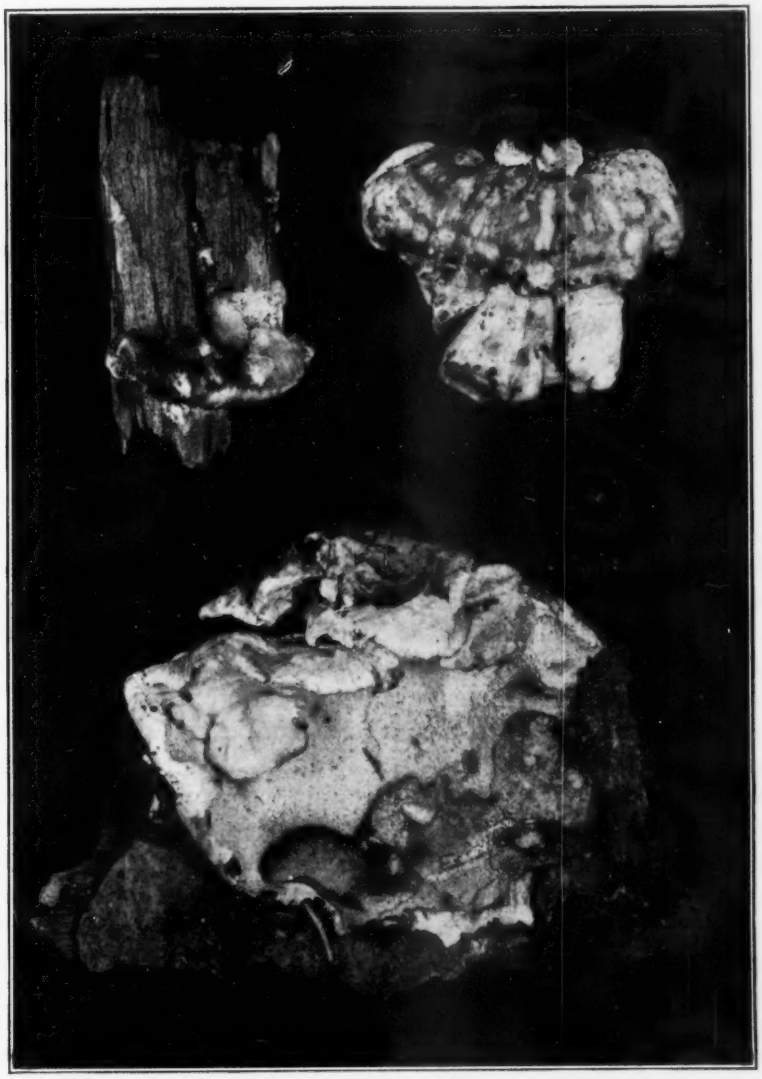

FIG. 3. Dried sporophores, upper and lower surface views, of *Polyporus balsameus* on *Abies balsamea*. The zonation of the upper surface is typical.

which completely and promptly riddle the fruiting body. There is frequently but a trace of the sporophore left attached to the tree.

The fungus develops annually. It bears a single layer of tubes distinct from the context. With the exception of the presence of cystidia and of certain variations in form and color, due mainly to local differences in habitat, Peck's description (5) may be used for this fungus:

Polyporus balsameus: "Pileus rather thin, corky, plain, about one inch broad, sessile or spuriously stipitate, slightly and unequally villose-tomentose, pale-brown, marked with lighter concentric zones; flesh white; pores short, minute, subrotund, the thin dissepiments acute, denticulate, white. Trunks of balsam trees, *Abies balsamea.* Adirondack Mts. August." (See Fig. 3, for the characteristic zonation.)

Isolation cultures from the early and late stages of rot in balsam fir were made on plain malt-agar slants. The 10-day-old cultures show white to grayish white aerial growth, fluffy at the lower end of the slant. Later this color changes to alutaceous or buff. Cultures 43 days old show a white to tan color in the lower slant while the upper slant shows a dark, reddish brown growth often somewhat horseshoe-shaped. The central area frequently shows small button-like groups of whitish hyphae which in older cultures are tipped a tan to brown color. The medium is usually stained a very dark brown color.

DAMAGE

Most of the balsam firs ranging in diameter from 3 to 18 inches, in the peninsula or Door-County region of Wisconsin were found to be infected with *Polyporus balsameus.* In the badly rotted trees the rot column was found to run from 6 to 12 feet up into the trunk in smaller trees and in lighter infections the rot column averaged 3 to 4 feet up the trunk. The rot column is usually circular in cross section of the trunk and tapers at the upper limit to a conical shape.

In the wet, "overflow" areas where rain and snow water or overflows from streams remain in hollows for considerable periods of time, many of the infected balsams were found broken by wind action either at or near the ground line or at points on the trunk ranging from 1 to 8 feet from the ground. Several trees were overturned or uprooted by the failure of the severely rotted supporting roots. These trees ranged from 4 to 20 inches in diameter.

Sporophores were most common on trees growing in very moist ground and were here found attached to exposed roots, in root crotches where frost cracks developed, and on scars or injuries to the root or root collar. They were rarely found on trees growing on slopes or well-drained ground.

In the Grand-Marais region of Minnesota conditions were observed that were very similar to those found in Wisconsin. In this region large areas of balsam and spruce trees were dead or dying from the attack of the bud worm. Many of the balsams showed balsam butt-rot and in the dead trees the rot developed rapidly, invading the sapwood and weakening the tree. As a result of this condition windfall and windbreakage were common, many of the trees being broken off at a point 2 to 6 feet from the ground. About 15 per cent of the balsam trees in this region showed butt-rot and these infected trees, in 3 to 5 years after death produced by bud-worm attack, were considered worthless by cruisers.

Windbreakage and windfall usually take place at from 1 to 3 years following the death of the trees.

The damage to potential pulpwood forests, where balsam fir is found in any appreciable amount, is reflected in the fact that second-growth stands of balsam, 50 to 60 years old, have the heartwood of the butt section and roots completely rotted. A high percentage of such infected trees are either uprooted or broken off by the wind.

SUMMARY

A brown butt-rot of *Abies balsamea* caused by *Polyporus balsameus* has been reported from Canada and the United States within recent years. The disease is a serious factor in the handling of pulp stands containing large percentages of balsam fir and particularly so in stands attacked by the bud worm, *Cacoecia fumiferana*. Symptoms of this heartwood disease are presented, including detailed, gross, and histological descriptions of the stages of decay.

Koch's rules of pathogenicity have been fulfilled in determining the fungus responsible for this butt-rot and a brief taxonomic description is given of *P. balsameus*.

It is pointed out that windbreakage and windfall are common types of loss resulting from the weakening of roots and butt sections of infected trees. This loss usually occurs at from one to three years following death of the trees due to the bud-worm attack.

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THE BLACK MOLD OF ONIONS, CAUSED BY *ASPERGILLUS NIGER* v. TIEGH.[1]

J. E. MACHACEK

During the autumn of 1928, the writer's attention was drawn to an unusual prevalence of black mold on imported Spanish onions. The epidemic appeared to be very severe and reduced considerably the market value of the vegetable. An investigation was made to determine the extent of the disease and to discover if there was any association of the causal organism with other, possibly pathogenic, bacteria and fungi.

Four shipments amounting to 40,000 cases of Spanish onions, imported from Valencia, Spain, were available for examination. These were examined when in the hold of the freighter, or while they were being unloaded into the dock warehouses, and also in cold storage. A casual examination showed the disease prevalent in each case, the percentage of diseased bulbs ranging from 10 to 100 per cent.

ISOLATIONS

An examination of the surface growth of the pathogene revealed the presence of *Aspergillus niger* v. Tiegh., but a more detailed examination of the shipments showed that some of the bulbs, though attacked by the above-named pathogen, also showed a rot, obviously of bacterial origin. Therefore, it was thought very probable that an association, such as had been described by the author in a previous paper (6), existed between the two or more pathogenes. Isolations substantiated the early microscopic examinations. Typical colonies of *Aspergillus niger*, *Fusarium* sp., *Erwinia carotovora* (Jones) Committee S. A. B., and a saprophytic Saccharomyces were obtained finally in pure culture. Of the above group only the Aspergillus and the bacterium proved pathogenic, the other two being saprophytes.

SYMPTOMS

From the examinations of many diseased bulbs it was concluded that in the majority of cases the initial invasion by the fungus began at the neck of the onion. In a few instances the diseased area originated at the base of the bulb, but the invasion of the neck, followed by a downward growth of the fungus, appeared to be the rule.

From the data available it was found that invasion of the bulbs begins at the time when some of the leaves of the plant met their death through wilting and breaking over, or after the death of the entire top at maturity,

[1] Due credit is here given to Prof. G. W. Scarth for valuable assistance given during the survey of the disease.

and then penetrates slowly into the interior of the bulb or spreads in the form of black streaks beneath the outer dry scales. In many cases it was found that, while there appeared to be no external evidence of the disease, yet, when the bulbs were cut in two, the central portion was blackened by the fungus, and usually a secondary rot had set in. Most of the bulbs examined were of peculiar construction, for it was found that an open space existed inside the bulb and extended from the base of the neck to varying depths within the bulbs. Usually this space was lined with the dried tops of the scales, and was, therefore, a suitable habitat for a weak parasite such as *Aspergillus niger*. This fungus initiated a minor rot in the bulbs, often a basis for a more destructive and extensive rot caused by *Erwinia carotovora*, which had probably invaded the plant in the wake of the above-mentioned fungus. (Fig. 1.)

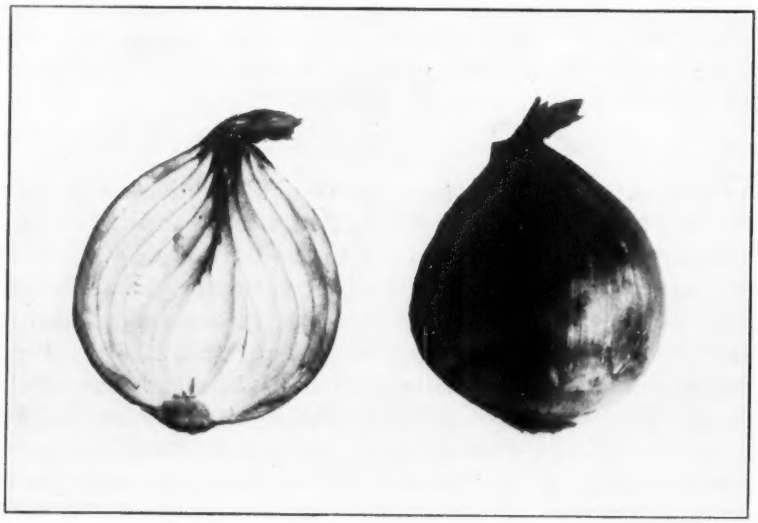

FIG. 1. Two halves of the same bulb showing open space in bulb. The fungus is present in dead scale tops.

Surface infections on the bulbs are characterized by the formation of shallow lesions on the outer scales, by the streaks of black fungous growth, and by discolored, irregular areas where the fungus had grown in bruises on the scales. (Figs. 2 and 3.) The surface infections, where there were no bruises, usually appeared as black streaks radiating from the base or the top of the onion bulbs, the most active growth of the fungus taking place along the veins of the bulb scales. (Fig. 4.)

Wayne (9) finds, in Ohio, that the disease does not confine its activities to mature bulbs, but also damages the seed and onion sets. He believes

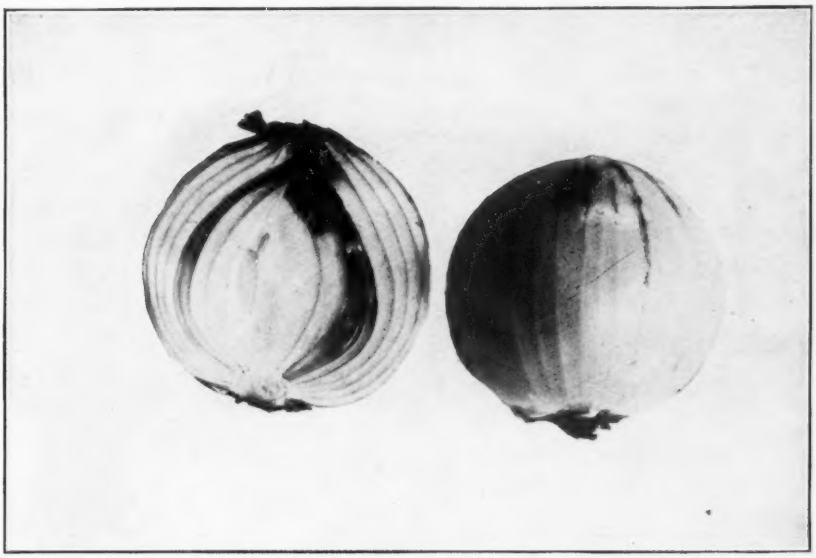

FIG. 2. Two halves of the same bulb, showing external streaking, as well as the bacterial soft rot induced by a condition shown in Fig. 1.

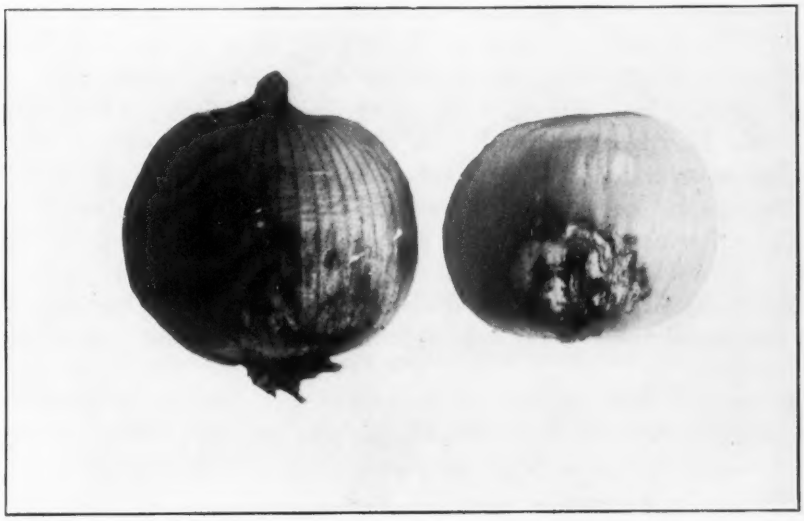

FIG. 3. Two types of surface rots caused by bruises followed by infection by *Aspergillus niger*.

(10) that the disease is carried by the seed and recommends remedies accordingly. Taubenhaus (8) finds that mature bulbs usually are most susceptible and that the infection is brought about through harvesting the

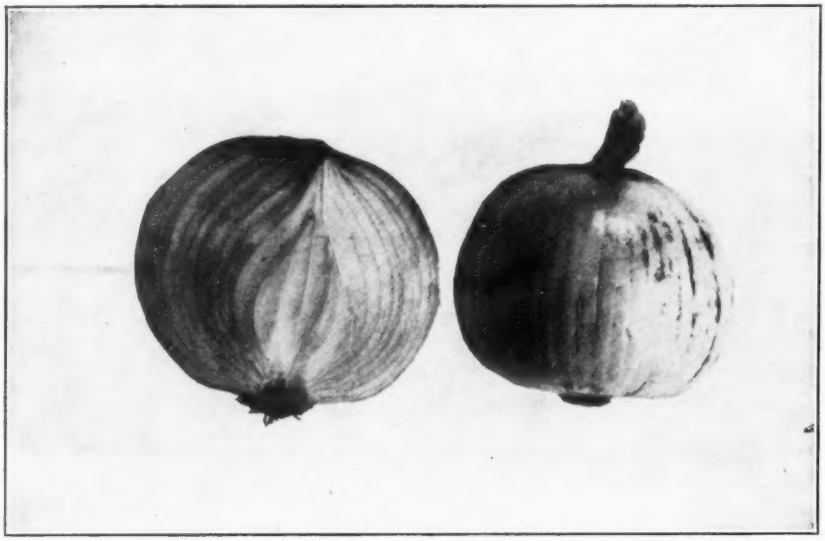

FIG. 4. Two halves of the same bulb showing outside streaking and healthy interior.

bulbs under unfavorable climatic conditions. He (8) gives the following characterization of the disease:

"The black mold first appears as blackened areas on the upper two or three layers of the bulb scales. When carefully examining these dark areas, one will find that they consist of deadened tissue, the surface of which has been blackened with loose spores of *Aspergillus niger*. As the diseased area increases, larger numbers of the bulb scales become involved and in time the entire onion shrivels and blackens and is then worthless."

The writer never has found the extreme condition mentioned above, for, though the market value of the onions was considerably reduced, unless the secondary, bacterial soft rot had made considerable progress, the bulbs were still marketable and could be kept so for a long period under proper storage conditions.

It has been observed that the amount of soft-rotted bulbs is in direct proportion to the number of bruised bulbs, this ratio being increased a good deal through the tendency of the bacterial rot to follow initial invasions of the bulbs by *Aspergillus niger*.

FACTORS INFLUENCING INFECTION

1. *Variety.*—Spanish onions appear to be attacked exclusively. This is probably due to the peculiar method of bulb maturing, with the top and center of the bulb remaining open to infection.

2. *Packing.*—Small cases and those which are loosely packed contain fewer diseased bulbs than large or tightly packed cases. In the former categories the amount of the disease was 10–50 per cent, while in the latter the amount was 40–100 per cent.

3. *Handling.*—Care in the handling of the bulbs during loading, transit, and unloading is essential. An examination showed that bruised bulbs are most liable to infection by Aspergillus, and also, in such bruises, the bacterial soft rot is almost certain to appear.

4. *Harvesting.*—Taubenhaus (8) finds that harvesting under proper conditions reduces the amount of infection considerably. He finds that during wet weather the onion bulbs become gorged with water and, in that condition, they are very susceptible to the disease. It is essential that harvesting should be done during dry weather whenever possible.

OTHER HOSTS OF THE PATHOGEN

Beille (1) observed that *Aspergillus niger* was able to induce a "stinking rot" of coffee grains.

Brooks, Fisher, and Cooley (2), working with fungi causing apple rots in storage and transit, found that *A. niger* was one of them.

Butjagin (3) reports that meat is susceptible to decomposition by *A. niger.*

Galli-Valerio and Rochaz-de Jongh (4) find that *A. niger* is pathogenic on mosquito larvae, but it is not practicable to use this fungus for the treatment of marshes and stagnant water where mosquitoes are breeding.

Hodgson (5) reports that *Sterigmatocystis* (*Aspergillus*) *niger* is able to cause an internal black "smut" of the white Adriatic fig.

Shapovalov (7) finds that *A. niger* can cause a tuber rot in Irish potatoes.

Taubenhaus (8) has been able to secure infection by the above organism on the pea, bean, tomato, carrot, pomegranate, peach, corn, and onion, and on bee larvae. In all cases the rot was severe.

There can be no doubt, therefore, concerning the ability of *A. niger* to cause disease in many other plants. As the fungus is cosmopolitan in its presence and can adapt itself to a wide range of conditions, it is probable that its weakly parasitic habits are the only explanation for its limitations as a phytopathogenic organism.

CONTROL

Wayne (10) suggests that, as a control measure, the onion seed should be sown by a drill to which an appliance is attached, capable of dripping a formaldehyde solution (one pint to 25–33 gals. water) into the seed bed along with the seed. As for the onion sets, he recommends soaking them for

six hours in a formaldehyde solution of the above strength or for the same length of time in a solution of powdered calcium hypochlorite (one-half pound in one gallon of water).

After infection takes place, the best means of preventing the disease from spreading through the bulb is to harvest the bulbs under dry conditions, dry them thoroughly after lifting, and then keep them in a cool, dry store room. Cold storage is recommended when the bulbs are to be kept for a prolonged period, as the fungus grows slowly under such conditions and the bulbs will remain in a reasonably good market condition for several months.

Care in packing and handling appears to be essential in order to retain the maximum market value of the bulbs. As bruises on the bulbs are almost certain to become infected, the prevention of these bruises by proper packing and handling is necessary.

SUMMARY

1. A bulb-rotting disease of Spanish onions induced by *Aspergillus niger* is discussed.

2. The fungus causes considerable discoloration of the bulbs, both on the outside and in the interior; and, while incapable of rotting the bulbs directly, causes a considerable decrease in the market value of the bulbs. It also enables the soft-rotting microorganism, *Erwinia carotovora*, to gain entry and begin a destructive rot of the bulb.

3. The interior of the bulb may be infected and rotted without any external evidence of the presence of the fungus.

4. Seed and set disinfection prevents most of the early field infection, but care in harvesting, handling during transit, and storing is necessary to the maintenance of the market value of the bulbs.

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STUDIES ON THE MYCELIUM OF *CRONARTIUM COMPTONIAE* ARTHUR ON *PINUS SYLVESTRIS* L.

W. G. Hutchinson

This study of the mycelium of *Cronartium comptoniae* Arthur has been undertaken in order to facilitate the determination of the presence of the rust upon Scotch pine in the absence of aecial fructification and other definite symptoms.

The characteristic symptoms manifested by trees infected with this rust are the swelling and cracking of the bark, the muscling of the wood, and the formation of fusiform galls (3) (4). In the early stages of the growth of the fungus, when these symptoms are not pronounced and there is no aecial fructification, it is difficult macroscopically to recognize the disease.

This study has been made on infected specimens of *Pinus sylvestris* L. collected by W. H. Snell in the neighborhood of Keesville, N. Y., in the summer of 1927.

Methods

Portions of the bark and wood were taken from over fifty different trees. This material was then cut into small pieces not over one-half inch square and placed in a formaldehyde-alcohol mixture made by adding 6 c.c. of commercial formaldehyde to 100 c.c. of 70 per cent alcohol (1). They were kept in this fixing solution about three months and then transferred to 30 per cent alcohol. They were then put through 20 per cent, 10 per cent, 5 per cent, and 2½ per cent alcohol and finally transferred to distilled water. Before sectioning, the pieces were placed in 5 per cent gum-arabic solution for about ten minutes.

In sectioning, staining, and clearing, the author has followed the methods outlined by Colley (2) in his study of *Cronartium ribicola*. The sectioning was done with an ether freezing microtome. The sections were cut from 5 μ to 20 μ in thickness. The thicker sections proved better for tracing the mycelium between the cells, but the haustoria could be much more easily seen in the thinner sections. Most of the sectioning was done in a radial plane, since the mycelium could be more readily traced through such sections.

The method of staining with safranin and *licht Grün*, as described by Colley, was employed. This method gave excellent results. Some of the sections were stained with the Pianeze 3B stain described by Vaughan (5). With this stain, also, the mycelium and haustoria could be distinctly seen.

MYCELIUM OF THE PARASITE AND ITS PARASITISM

The mycelium of *C. comptoniae* has been found very irregular in outline, varying in width from 3.6 μ to 6.2 μ. It frequently appears swollen, forming characteristic bulges, is septate, and distinctly vacuolate. Each cell of the mycelium is uninucleate.

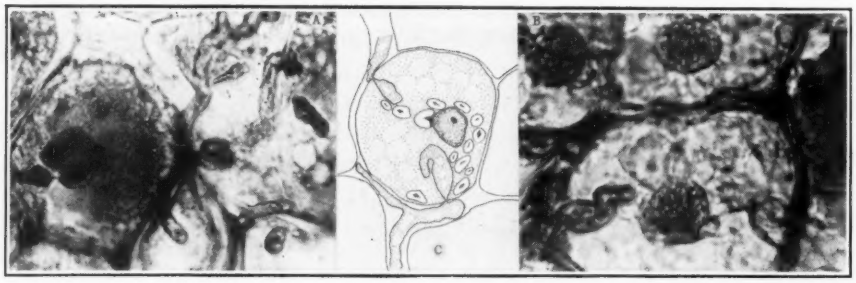

FIG. 1, A. Cells from bark of *Pinus sylvestris* infected with haustoria of *Cronartium comptoniae*. Note nucleus dented by a haustorium. ×420. B. Haustorium of *Cronartium comptoniae* showing typical bulge and bending toward the nucleus of the host cell. ×500. C. Camera-lucida drawing of cells from bark of *Pinus sylvestris* penetrated with haustoria of *Cronartium comptoniae*. Note the haustorium which has grown toward the nucleus and then has bent backward and grown toward the cell wall. Note also the numerous starch grains in the cell. ×320.

Numerous haustoria enter the cells of the bark. At the point where a haustorium passes through the wall of the host cell, it is noticeably constricted (Fig. 1, C). At this point of constriction, the haustorium measured 1 μ to 2 μ in width. The portion of the haustorium within the cell averages 5.2 μ in width and, in some parts where bulges occur, it may be as much as 8 μ in width. After penetrating the cell for some distance, the haustorium characteristically bends toward the nucleus (Fig. 1, B). Several cases were observed where the nucleus had been dented by the haustorium (Fig. 1, A). No cells have been noted in which the haustoria were branched. In a few cells the haustoria were observed growing into the cell toward the nucleus and the bending backward and toward the cell wall (Fig. 1, C). What causes this reaction it is difficult to determine. It is perhaps caused by some repelling influence in the nucleus or by some chemical substance in the cell which is antagonistic to the haustorium.

The mycelium of this fungus may be distinguished in general from that of other fungi occurring on *Pinus sylvestris* by its large size, the presence of large haustoria, and the characteristic bulges which occur chiefly on the haustoria. Its growth is intercellular. The cells adjacent to the mycelium are often pushed widely apart by the hyphae (Fig. 1, C). This probably accounts for the swelling of the bark.

Haustoria have been observed in the parenchyma cells of the cortex and phloem, in the sieve tubes, and in the ray parenchyma. The parenchyma cells of the phloem and the ray parenchyma are the principal cells attacked. In very few cases has the mycelium been found in the xylem. When it has been found there, no definite haustoria were evident; the mycelium appeared to be inside of the tracheid cells and to be growing through the bordered pits.

The cells entered by the haustoria do not appear to be greatly damaged by the fungus. In most cases they are somewhat more vacuolate than the normal cells. Most of them contain starch grains or oil globules (Fig. 1, C).

IDENTIFICATION BY MYCELIUM

Pinus sylvestris is subject to several rusts besides *Cronartium comptoniae*, namely, *Cronartium pyriforme* (Peck) Hedgc. & Long, *Cronartium cerebrum* Peck, and the Woodgate rust (3). The fact that *C. cerebrum* and the Woodgate rust have globose galls removes any possibility of confusion between *C. comptoniae* and these rusts. From microscopic study of mycelium, however, it is yet impossible to distinguish between any of these fungi. Until a comparative study of the mycelium of *C. comptoniae* and that of the other rusts has been made, the positive identification of this fungus by its mycelium is impossible. In certain cases, however, the method of identification outlined above is of value. The mycelium around the aecia has been found to correspond with that in suspected cases.

SUMMARY

1. The method of double staining of the mycelium of *Cronartium comptoniae* with safranin and *licht Grün* has been followed and found to give excellent results.

2. The mycelium of *Cronartium comptoniae* is characterized by its large size and by the possession of bulged haustoria.

3. The mycelium occurs in the parenchyma cells of cortex, phloem, and medullary rays, in the sieve tubes, and occasionally in the tracheid cells of the xylem.

4. The fungus parasitizes the cells of *Pinus sylvestris* by growing between and separating them and also by penetrating the cells with its large haustoria.

5. A comparative study of the mycelia of all the rusts occurring on *P. sylvestris* is necessary before a definite determination of the fungus by its mycelium is possible, although this method of identification is of value in certain cases.

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A ROOT AND BUTT ROT OF CONIFERS CAUSED BY *POLYPORUS CIRCINATUS* FR.

ERNEST E. HUBERT

Attention already has been directed to the work done in the forest and by means of cultures in the laboratory, in associating *Polyporus circinatus* Fr. with a white-pocket rot of roots and butt sections of conifers (2) and the comparison of pure cultures of this fungus with cultures of *Trametes pini* (Brot.) Fr. (2). During the past field season, the fungus was observed attacking the roots and butt sections of living western white pines, *Pinus monticola* Dougl., in northern Idaho. Acting as a wound fungus, it enters through injuries to the roots or root collar and produces a serious heart rot of the butt of many trees. It appears to be more prevalent in the white-pine areas where basal fire scars are common and invariably one may expect in such stands a considerable percentage of butt rot, partly caused by the above fungus and partly produced by *Polyporus schweinitzii* Fr. The fungus has also been collected in association with the typical rot in Engelmann spruce, *Picea engelmanni* Engelm., Douglas fir, *Pseudotsuga taxifolia* (Poir.) Britt., western hemlock, *Tsuga heterophylla* (Raf.) Sarg., and western larch, *Larix occidentalis* Nutt. The fungus associated with the typical rot has also been collected on *Picea mariana* (Mill.) B., S., and P. in northern Minnesota.

Previous to these observations and laboratory tests, this rot was attributed to *Trametes pini* and was assumed to be a butt-rot development of the ring-scale fungus differing from the normal development of this rot, which is commonly found in the main trunk and top of the tree.

Faull, in 1922 (1), reported a root and butt rot in spruce, hemlock, and eastern white pine caused by *Polyporus tomentosus* Fr., a synonym for *P. circinatus*. The description and habit of the sporophore correspond to those of *P. circinatus* and the rot is described as a white-pocket rot. He states that in the Otter district of Ontario a high percentage of the white pine is affected and results in considerable culling of the butt logs.

In 1927, Solovieff (5) reported a rot in spruce which he attributes to *Polystictus triqueter* Fr. This fungus is considered by mycologists to be very near if not identical with *P. circinatus* but Solovieff retains the former name because of differences in the macroscopic structure of the fruiting bodies of the two and differences in the rot descriptions. In western Russia this fungus causes considerable damage to spruce trees, the rot extending a distance of about 10 feet into the trunk from the base of the tree and for some distance into the roots. Because of the weakening of the roots and of

the butt section due to the rot, the infected trees are said to be very subject to windfall.

This is the second fungus the rot of which has been differentiated (3) from that caused by *Trametes pini*. The first fungus of this type, *Polyporus anceps* Pk., produces a white-pocket rot known as red-ray rot in the heartwood of living western yellow pine, *Pinus ponderosa* Laws., and in the heartwood and sapwood of the dead tree and its parts (4). For some time past, scalers of the Inland Empire region have called this typical top rot, "red-ring rot," and believed it to be caused by *T. pini*. Red-ray rot is a common rot of the slash and débris of western yellow pine found on logging areas in the above region.

DESCRIPTION OF DISEASE

The early-stage discoloration of red root rot caused by *P. circinatus* appears as a dark, reddish brown band surrounding the white-pocket or late decay areas. The darker color is one of the main characters distinguishing the early stage of this rot from that caused by *T. pini*. The discoloration accompanying early decay caused by *T. pini* is pinkish, reddish, or purplish, but never as dark in color as in red root rot. The wood, though darkly colored, is firm and shows no signs of pocket formation at this stage of development. Cultural studies indicate the presence of fungus threads in the discolored, early rot areas.

Numerous long, narrow and elliptical pockets filled with white fibers characterize the late stage of decay. The pockets have pointed ends similar to those found in ring rot, and these frequently run together in the direction of the grain of the wood. The wood between pockets is of a dark brownish color similar to the early rot discoloration. Unlike red ring rot, no zone lines appear in the rot and no black flecks have been noted in the pockets similar to those found in spongy sap rot, caused by *Fomes annosus* Fr., and in spongy root rot caused by *Poria subacida* Pers. (4).

Histological studies of red root rot give further evidence of the differences between this rot and that caused by *T. pini*. The hyphae of *P. circinatus* in *Picea mariana* are not constricted where they pass through the cell walls and the bore holes in the walls are considerably larger in diameter than the hyphae. Most of the hyphae run lengthwise of the wood cells; very few are observed penetrating many walls in a direct line. The hyphae of *T. pini* usually are constricted where they pass through small bore holes in the cell walls, show buckles, and penetrate several walls in a direct line.

The cultural characters of *P. circinatus* on malt-agar media differ sufficiently from those of *T. pini* on the same media to make possible their successful use in the identification of the two rots. The cultures of the former are of a much darker brown and do not show the cottony aerial growth so

common to cultures of *T. pini*. *P. circinatus* produces a subsurface growth in cultures and the surface growth is somewhat adpressed. The agar immediately beneath the mycelial mat is dark brown when viewed from the side of the tube opposite the slant or from the under side of the petri dish. The aerial hyphae of *T. pini* are light orange yellow to cinnamon buff.

SPOROPHORES

The sporophores of *Polyporus circinatus* are commonly found developing on the forest duff or on the ground some little distance away from the trunk of the infected tree. In this respect, it resembles in habit the sporophore of *P. schweinitzii* which is most commonly found on the ground. *P. schweinitzii* is sometimes found on infected logs or stumps and attached to the butt section of trees. In this environment it is truly applanate and shelf-shaped, departing from the usual circular, depressed form and central stipe. *P. circinatus* has not yet been collected on the trunk of the host but has been found attached to the buried roots by means of the mycelial strands which penetrate the duff and soil. The sporophores vary considerably in size and color with changes in environment, being larger, more regular in form, and brighter in color on moist sites and smaller, somewhat irregular in form, and darker on drier and more exposed areas. It is not found in very dry situations, since it is favored by a fairly moist environment. The sporophores may easily be mistaken by woodsmen for small or imperfectly developed sporophores of *P. schweinitzii*.

Little is known regarding the method of infection. The field observations made in Minnesota, Idaho, Washington, and Montana, indicate the fungus to be a wound organism, entering the roots through injuries of some kind and developing in the heartwood as a heart-rot agency. Correlation between fire scars at the base of the tree and on exposed parts of the roots with the entrance of this fungus has not been established, although the relationship between red root rot and basal fire injuries seems to be fairly constant.

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INACTIVATION OF THE TOBACCO-MOSAIC VIRUS BY HIGH PRESSURES

N. J. Giddings, H. A. Allard, and B. H. Hite

Some years ago (1915–1920), Giddings and Hite were conducting experiments to determine the effects of high pressures on various enzymes, and, at the suggestion of the senior author, in October and November, 1919, a little research was conducted on the tobacco-mosaic virus. Because of the present great interest in virus diseases and the fact that these experiments indicate possibilities of another method of learning something about them, it has seemed desirable to report on this research. The equipment and methods used have already been described in detail.[1]

Freshly expressed juice from mosaic-diseased tobacco plants was sent from Washington, D. C., to the West Virginia Agricultural Experiment Station, where the pressure equipment was located. This juice was immediately placed in an ice box where all of the samples were kept, except when they were in the press, until they were returned to Washington. All of the juice, both for use in the press and that held as control samples, was kept in block-tin tubes from the time it was received at the West Virginia Agricultural Experiment Station until it was used for inoculations in Washington.

In view of the fact that some of our other work was conducted with the materials at temperatures of 40 to 60° C., it may be well to state that all work with the tobacco-mosaic virus was at room temperatures (20–22° C.).

In all inoculation tests, young, rapidly growing Maryland Mammoth tobacco plants were used, as previous experience had shown that young plants at this stage could be used to best advantage. Ten plants were employed in each test. The results are given in table 1.

It will be noted that the twenty control plants all remained healthy; the thirty plants inoculated with the untreated mosaic juice showed twenty-six mosaic plants; the forty plants inoculated with mosaic juice which had been subjected to 75,000 lbs. pressure showed thirty-one mosaic plants; and that mosaic symptoms did not develop in any of the plants inoculated with mosaic juice which had been subjected to pressures of 130,000 lbs. or more.

The pressure required to inactivate or destroy this virus is higher than the pressure death point of *Bacillus prodigiosus* (Ehrenberg) Flugge; *Bacterium lactis aerogenes* Huppe; *Streptococcus lacticus* Kruse; *Bacillus typhosus* Eberth; *Bacillus diphtheriae* Loffler; *Saccharomyces cerevisiae*

[1] Hite, B. H., N. J. Giddings, and C. E. Weakley, Jr. The Effect of Pressure on Certain Microorganisms Encountered in the Preservation of Fruits and Vegetables. W. Va. Agr. Exp. Sta. Bul. 146. 1914.

TABLE 1.—*Summarized results which show the inactivating influence of high pressure for different periods of time on mosaic-tobacco extract*

Source of extract	Separate trials	Treatment		Total plants inoculated	Total plants infected
		Pressure	Time		
	No.	Pounds	Days	No.	No.
Healthy tobacco	2	0	0	20	0
Mosaic tobacco	3	0	0	30	26
do	2	75,000	7	20	15
do	2	75,000	5	20	16
do	2	130,000	1	20	0
do	3	135,000	2	30	0
do	1[a]	200,000	½	10	0
do	5	200,000	2	50	0
do	2[b]	200,000	7	20	0

[a] This sample was given additional treatment of ½-day at 100,000 pounds.

[b] These samples were given additional treatment of 15 hours at 100,000 pounds.

Meyer; and *Saccharomyces albicans* Reess, but lower than that of the spore-forming organism, *Bacillus subtilis* F. Cohn. It may also be noted that the enzyme zymase is inactivated or destroyed by lower pressure than that required to destroy the tobacco-mosaic virus, while pepsin is inactivated or destroyed at pressures which would seem likely to approximate those required for the tobacco-mosaic virus if its "pressure death point" had been more closely determined.

It is clearly evident that the higher pressures were effective in destroying or inactivating the virus of the tobacco mosaic and it is reasonably certain that the time-pressure line between 75,000 lbs. and 130,000 lbs. would yield further data of considerable interest. It is also highly probable that similar studies with other viruses would assist materially in their classification, by bringing out their reaction to this test.

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PHYTOPATHOLOGICAL NOTE

The relation of moisture to stomatal infection.—At the meeting of the American Phytopathological Society, in December, 1928, a paper was presented entitled "Studies on the influence of environment on infection by certain bacterial plant parasites." An abstract of the paper was published in Phytopathology.[1] This paper presented as a new discovery the fact that many more stomatal infections are obtained when plants are held in the damp chamber for 24 hours before spraying with inoculum than when they are inoculated at once on being placed in the damp chamber.

This fact has been known and utilized for many years in the Laboratory of Plant Pathology in the United States Department of Agriculture, in Washington; in fact, this has been the routine method of procedure in producing stomatal and waterpore infection.

As long ago as 1905 Dr. E. F. Smith[2] published directions for spray inoculations as follows:

". . . Stomatal infections may be secured by subjecting the plants to conditions similar to those occurring in nature on dewy nights or during heavy fogs or prolonged rains, i.e., by placing the potted plants on wet sand, atomizing thoroughly with sterile water and covering with tall, roomy bell-jars. The experiment should be undertaken in a cool rather than a warm house. When the right conditions have been obtained, moisture covers the surface of the plant in tiny drops which do not evaporate. The bell-jar may now be raised and the plant again atomized lightly with sterilized water containing the bacterium. The best time to do this is late in the afternoon, so as to take advantage of the cooler night temperature. When the bell-jar is returned, which should be immediately after spraying, it should be covered with cloth or paper to protect from the light. Usually bell-jars should be removed at the end of twenty-four hours, but exceptionally they may be left on thirty-six to forty-eight hours, if not exposed to the sun. . . ."

It will be seen that although Dr. Smith does not specify the number of hours for this preliminary treatment, he describes the condition of the plant ready for inoculation for which approximately that length of time in a damp chamber is required.—MARY K. BRYAN.—*Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.*

[1] Riker, A. J. Studies on the influence of environment on infection by certain bacterial plant parasites (Abst.). Phytopath. **19**: 96. 1929.

[2] Smith, E. F. Bacteria in relation to plant diseases. **1**: 108. Washington, D. C. 1905.